DISSERTATION ON

ETIOLOGICAL PROFILE OF INFECTIOUS FEVER -BACTERIAL AND PARASITICAL

Submitted to

The Tamil Nadu Dr. M.G.R. Medical University

FOR

M.D. DEGREE EXAMINATION

BRANCH – IV (MICROBIOLOGY)

THE TAMIL NADU DR. M.G.R MEDICAL UNIVERSITY

CHENNAI, INDIA

SEPTEMBER 2006

CERTIFICATE

*This is to certify that the dissertation entitled "**ETIOLOGICAL PROFILE OF INFECTIOUS FEVER - BACTERIAL AND PARASITICAL**" is a bonafide work done by **Dr. G. THANGAMATHI**, postgraduate Institute of Microbiology, Madras Medical College Chennai, under my guidance and supervision in partial fulfillment of the regulation of The Tamil Nadu Dr. M.G.R. Medical University for the award of M.D. Degree, Branch-4 (Microbiology) during the academic period of August 2003 to September 2006.*

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ACKNOWLEDGEMENT

First of all I owe my thanks to Almighty God for showering his blessing on me and shwoed me the right path and smooth way.

I express my sincere gratitude to the Dean, ***Dr. Kalavathy Ponniraivan,*** **M.D.,** for permitting me to use the resources of this institution for my study.

At the outset, I wish to express my gratefulness to ***Dr. A. Lalitha,*** **M.D., DCP.,** Director and Professor, Institute of Microbiology for allowing me to take this study and thrusting her whole hearted support all along this study.

I would like to express my heartful gratitude to former Director & Professor ***Dr. T.S. Vijayalakshmi*** **M.D.,** for her encouragement and able guidance during my study.

I express my sincere gratitude to Vice Principle ***Dr. S. Geetha Lakshmi*** **M.D.,** for her valid comments and supports to this study.

I feel a great pleasure to take this opportunity to express my sense of gratitude to my respected guide ***Dr. Sumathi Gnanadesikan*** **M.D., Ph.D.,** Additional Professor, Institute of Microbiology, for her guidance, constant encouragement with expert advice and meticulous care throughout my study.

It is my privilege to express my profound gratitude and immense thanks to my Additional Professors ***Dr. A.S. Shameem Banu*** **M.D.,** ***Dr. Sasireka*** **M.D.,** and ***Dr. Kalavathy Victor*** **M.D.,** for their valuable assistance in this study.

I am greatly indebted to ***Dr. T. Latha*** **M.Sc., Ph.D.,** for her untiring efforts and infectious enthusiasm. Her friendliness, care and her helping hand was instrumental in completing this study.

I also extend my whole hearted gratitude to Assistant Professors
Dr. Sujatha Varadarajan ***M.D., Dr. Kaveri*** **M.D.,** ***Dr. Indumathi*** **M.D.,** and
Dr. K. Radhika **M.D.,** for their guidance.

I express my prayers and love towards my family members and parents for their encouragement prayers, patience, support and advice without which of would not have been able to pursue the course of my study.

Last but not the least, my sincere thanks to all the patients who co-operated for his study and without whom this study would have been impossible.

CONTENTS

INTRODUCTION

The concept of "Fever of unknown", "undiagnosed", "unexplained origin" or "pyrexia of unknown origin" evolved in the medical literature from 1950. FUO is a challenging medical problem. Fever is defined as an elevation of core temperature above normal, i.e. > 37.8°C due to resetting of the thermoregulatory centre in medulla. The cause can vary from minor brief illness to life threatening infections, malignancy or autoimmune disease[45].

The spectrum of FUO seems to be determined by geographic and economic factors, and it appears to change in time. Categorisation of fever including the duration and the presence of specific localising signs have been difficult because of areas of overlap.

In most patients with fever lasting one or two weeks, the underlying disorder is soon discovered or the patient recovers spontaneously. In other patients however, fever continues for two or three weeks during which time physical examination, chest x-ray films, blood tests and routine cultures do not reveal the cause of fever. In these cases the provisional diagnosis of fever of unknown origin (FUO) is made.

The term "Fever of unknown origin" and pyrexia of unknown origin" are interchangeable. Immuno competent patients were included using criteria for FUO according to Petersdorf and Beeson PB (1961) [87]

The symptoms and signs may be highly variable some have trivial symptoms and others may be incapacitated by debilitating chills, rigors, sweats and dehydration. Certain diseases have been known to produce characteristic pattern of fever, notably in malaria, brucellosis, rickettsial infection and enteric fever.

Fever can be of different types such as intermittent, remittent or continuous. An exaggerated circadian rhythm that includes a period of normal temperature on most days is termed as "intermittent fever" extremely wide fluctuations may be termed as "septic fever". "Remittent fever" varies by more than 0.5°C during the course of the day but does not return to normal. A "sustained fever" is persistent and does not vary by more than 0.5°C/day. Relapsing fever should be distinguished from infectious diseases with a tendency to relapse with intervals of normal temperature. A "biphasic fever" indicates a single illness with two distinct periods of fever over one or more weeks. The clinical characteristics of drug-induced fevers are highly variable, despite the common misconception that they are usually low grade fevers with relatively little variations from peak to trough and relatively low pulse rate. Individual variations and the common use of analgesics with antipyrexial effects confuse the diagnosis.

Fever is a common manifestation of various infections with a wide range of severity. Benign febrile diseases usually respond well to appropriate antibiotics and are not life threatening.
The causes for most of the FUO can be included under five simple categories [99].

1. Infections
2. Malignancies

3. Connective tissue disease

4. Miscellaneous conditions including fictitious fever, drug induced fever and cryptic haematomas

5. Undiagnosed.

Within the first three categories infections predominate. Infectious diseases are the commonest and important causes of FUO and they are often curable. Severe bacterial infections if untreated may have significant morbidity and mortality as in the case of fever in meningitis, pneumonia and tuberculosis. Hence more importance should be given for diagnosing the cause of any prolonged fever.

Various studies has been done on FUO in other countries, but only limited studies have been done in India. Such a study will help in finding out the etiological agents causing FUO and to define the changing pattern of the causative agents in a tertiary care hospital in Chennai. The presence of advanced diagnostic techniques have helped in finding out relatively higher percentage of infections. Hence this study was undertaken, restricting it to FUO of bacterial and parasitic origin.

AIM OF THE STUDY

1. To study the percentage of microbial infections in FUO cases attending Govt. General Hospital, Chennai.
2. To identify the bacterial agents causing fever.
3. To findout the parasites causing fever.
4. To detect the presence of leptospiral antigen by DFM, culture and PCR and antibody detection by serological methods.
5. To compare the blood smear study with rapid diagnostic test for the identification of malarial parasite.
6. To carry out blood smear study for the detection of filariasis, babesiosis, leishmaniasis and toxoplasmosis.
7. To compare the conventional method with rapid test for antibody detection in enteric fever.
8. To identify the etiological agents causing urinary tract infection, pneumonia, bacteremia and to determine their antibiotic susceptibility pattern.
9. To carry out serological tests for antibody detection in typhus fever and brucellosis.

REVIEW OF LITERATURE

Febrile episodes are common, often transient and often due to obvious cause. In a few cases, fever is persistent and the cause is not easily diagnosed. Such episodes are FUO.

DEFINITION OF FUO

Fever of unknown origin (FUO) was defined by Petersdorf and Beeson[87] in 1961 as (1) temperatures of > 38.3°C (> 101°F) on several occasions, (2) a duration of fever of > 3 weeks and (3) failure to reach a diagnosis despite 1 week of inpatient investigations.

Various authors all over the world, have performed studies on classical FUO from 1952 which is depicted below :

CAUSES OF CLASSICAL FUO IN ADULTS (FROM MILLER & DURACK DT 1994)[49,73]

Author & year of study	*No.of cases*	*Infections*	*Neoplasm*	*Collagen disease*	*Miscellaneous*	*Undiagnosed*
Petersdrof (1952-57)	100	36%	19%	13%	25%	7%
Jacoby (1957-71)	128	40%	20%	15%	17%	8%
Howard (1969-76)	100	37%	31%	19%	8%	5%
Larson (1970-80)	105	31%	31%	16%	10%	12%
Knockaert (1980-89)	199	22.5%	7%	21.5%	26.5%	22.5%
Sharma (1974-89)	150	50%	21%	9%	15%	5%
Kazanjian (1984-90)	86	32.5%	24.4%	16.3%	17.5%	9.3%
Kejariwal D (1998-2000)	100	53%	17%	11%	5%	14%
Kucukardaly (2002)	82	59%	11%	7%	2%	21%

The largest study is by Knockaert DC (1992)[54] spanning nine years from 1980-1989. In all the studies except in Knockaert DC (1992)[54] infection is the major cause of FUO. The spectrum of diseases found in several series examining FUO shows some variation, but overall, infections continue to be the most important cause of FUO accounting for about 53% followed by neoplastic lesions, collagen vascular disorder and other rare illness[51]. The spectrum of diseases causing FUO not only seems to be determined by geographical factors, but also appears to change with time[99]. Infections formed the majority of causes of FUO. A further breakup of the infectious causes are as follows:

LISTING OF INFECTIONS CAUSING FUO
(FROM MILLER AND DURACT DT 1994) [49,73]

Infection	*Location / Year / No.of patients*					
	Connecticut 1952-57 100	***Washington 1970-80 105***	***Belgium 1980-89 199***	***India 1974-89 150***	***Rhode id 1984-90 86***	***Spain 1968-81 133***
Abscess	22%	20%	6%	5%	23%	3%
Mycobacterium	11%	5%	5%	25%	5%	11%
Endocarditis	5%	0%	2%	3%	5%	2%
UTI	3%	3%	1%	0%	0%	1%
Viral	0%	4%	5%	0%	5%	0%
Protozoal	0%	1%	0%	9%	2%	2%
Brucellosis	1%	0%	0%	0%	0%	3%

The commonest causes of bacterial and parasitical etiologies of classical FUO are depicted below[53]

Bacteria	**Parasites**
- *Salmonella typhi and paratyphi* - *Leptospira sp.* - *Mycobacterium tuberculosis* - *Brucella sp.* - *Klebsiella pneumoniae* - *Staphylococci sp.* - *Rickettsiae sp.*	- *Plasmodium sp.* - Filarial worms - *Babesia microti* - *Leishmania donovani* - *Toxoplasma gondii* - *Entamoeba histolytica*

TYPHOID

Typhoid fever is an important public health problem in tropics and developing countries. It is endemic in India. It remains an important cause of morbidity and mortality (upto 500 / year) in many developing countries. The disease often does not show a specific clinical picture, especially early in the disease and can be confused with other febrile illness such as dengue fever, malaria and leptospirosis, which are often prevalent in the same area. Typhoid fever is transmitted via faeco-oral route and the incubation period is 10-14 days[47].

A retrospective study suggests that cryptic plasmid bearing strains of *Salmonella typhi* were associated with clinical and bacteriologic relapse in typhoid fever[62]. Typhoid fever is difficult to differentiate from other causes of infection such as malaria because their signs and symptoms often overlap. Febrile patients are often treated for malaria and typhoid fever simultaneously[81]

DIAGNOSIS

Culture

Diagnosis can be done by Blood cultures, which when done during the 1st week or 10 days of illness will give a positivity of 90%. By the third week, chances of obtaining a positive blood culture is reduced to half. Culture methods for identification of *Salmonella sp.* from blood and stool often takes several days and can be falsely negative if antimicrobial agents have been previously administered.[88]

Serology

By 2-mercapto ethanol, IgM antibodies can be inactivated in modified widal test, the agglutination would be brought about only by specific IgG, while in the conventional widal test agglutination is due to specific IgG and IgM. The difference in the titres indicates specific IgM class of antibodies which is the hallmark of recent infection. If conventional widal test and modified widal test are simultaneously done, one can be definite about the diagnosis of enteric fever[82]. Since last 50 years drug resistances in *Salmonella typhi* has been known to the world. In India chloramphenicol resistance in *Salmonella typhi* was first reported from Kerala. It has been an ever increasing problem in developing countries specially in India. Drugs should be prescribed in accordance with the sensitivity pattern[118]

The dipstick assay applied to a single serum sample gives a quick result (3 hours). Testing of paired sera could increase the sensitivity of the assay and allow demonstration of sero conversion providing stronger evidence of disease; however the results would not be known for several days[36]. Evaluation of a simple and rapid dipstick assay for the diagnosis of typhoid fever has been done in Indonesia[39].

Molecular techniques

The Polymerase Chain Reaction (PCR) assay, is a rapid diagnostic method for detection of *Salmonella typhi* infection in blood specimen from patients with typhoid fever, particularly when results of standard culture assays are negative[65]

In order to develop unique PCR primers to detect *Salmonella typhi*, ribosomal RNA genes from *Salmonella typhi* (Rawlings) were cloned in PUC18. This pair of primers therefore have the potential for development into a diagnostic tool for the rapid diagnosis of typhoid fever[130]

Salmonella enterica sero type *Paratyphi A* which had earlier been reported less frequently (1-15%, 3-17%) from cases of enteric fever, has shown an increasing trend since 1996 in North India[68]. Chandel

et al[10] attributed this dramatic increase in incidence of enteric fever by *S. paratyphi A* to wide spread use of vaccines and quinolones against *S. typhi* in the past decade.

LEPTOSPIROSIS

Leptospirosis is the most prevalent zoonosis in the world with varying clinical manifestations. Since it causes loss of cattle and man it poses a serious public health problem. It is still under reported due to lack of diagnostic facilities[77]

Leptospirosis has been recognised as an important emerging disease in the 1980's in Andamans, Tamilnadu and Kerala. It may also occur endemically in trophical countries, showing seasonal incidence following heavy rain fall[50]

Human leptospirosis occurs from direct or indirect exposure to urine of infected animals. Persons of all ages and sexes are susceptible. Adult men are more frequently infected. Incidence is maximum during the rainy season (Oct. - Dec.) [47]. The fever pattern in leptospirosis is biphasic in nature though the pattern is not consistent. It can present in the icteric or anicteric forms and the former has got a bad prognosis because of hepato renal failure. The commonest serovars in Tamilnadu are *Ictero haemorrhagiae, Patoc, Grippotyphosa, Hebdomadis, Louisiana* and *Pomona*[93].
Incidence of co-infection among confirmed cases of leptospirosis has been reported by Ramakrishnan et al [92] in associations with enteric fever 17% and malaria 5%. Certain occupational groups, including rice-farmers, fishermen, sugarcane workers, sewer workers, and military personnel are considered to be at increased risk of leptospirosis[122]. Epidemic leptospirosis associated with pulmonary haemorrhage, renal failure and jaundice predominates in the urban setting, where baseline clinical immunity in humans is likely to vary. Generally transmission and clinical expression of the infection varies in different environmental and socio-economic contexts[70]

In 1966, a human leptospirosis case was reported in Delhi. Since then it has been reported from Madras, Kerala, Maharashtra, Uttar Pradesh and Andhra Pradesh[108]. The persons handling animals are usually at a higher risk. It should be suspected more frequently in patients of FUO with or without Jaundice, especially with history of animal contact and diagnosis can be established by laboratory investigations. Leptospirosis is a seasonal disease, which starts at the onset of spring, reaches its peak in summer and declines as winter sets in[18]. Onset of fever associated with rapid rising of azotemia or pulmonary hemorrhage and hemoptysis after a flood or the rainy season should made one think of leptospirosis[2].

Outbreaks of febrile illness with jaundice and myalgia have been reported from the penal settlements of Port Blair during the first half of this century. These outbreaks had a definite seasonal pattern with maximum number of patients being seen towards the end of monsoon. Every year since 1988, outbreaks of febrile illness with haemorrhagic manifestation have been reported in young adults mainly engaged in agricultural activities from North Andaman. The outbreak had high fatality rate in the range of 10 to 50%[97].

Anicteric leptospirosis is a self limited illness that occurs in 90% of the cases. The most common physical finding is conjunctival suffusion along with fever, severe muscular pain, vomiting and abdominal pain. The symptoms are prominent for 4 to 7 days i.e. during the septicemic stage and end of this phase, defervescence occurs and the patient is afebrile for a day or two, before the immune phase begins. The immune phase is characterized by the presence of circulating antibodies, for 4 to 30 days. Involvement of other organs occurs in this phase. Aseptic meningitis is the hallmark of the immune phase[94]. Icteric leptospirosis or Weil's disease is seen in about 10% of the cases. The biphasic course of the disease in obscured by severe and persistent fever, jaundice and azotemia. Severely jaundiced patients are the ones more likely to exhibit renal failure, haemorrhage and cardiovascular collapse. Thrombocytopenia occurs in most patients with renal failure[21].

DIAGNOSIS

Leptospires can be isolated from blood and CSF during first 7-10 days of illness and from urine during the 2nd and 3rd week of illness.

Diagnosis can be made either by demonstration of the organism or serological test and molecular techniques.

Dark Field Microscopy (DFM)

This is invaluable for examining the growth of culture and antigens, but as a diagnostic tool it has severe limitations. Nevertheless, it is still widely used in diagnosis[25]. During leptospiraemia, leptospires may be found by dark field examination of blood or urine after the first week of disease[107].

Staining of leptospires

Fontana’s method for films and levaditis method for sections are classical methods of silver staining for leptospires. In Fontana's method, the leptospires stains dark brown against yellow brown background. The various other silver stains with slight modifications are warthinstarry technique with Faine's and Young's modification[13].

Culture method

Leptspiraemia occurs during end of first week. Blood should be taken as soon as possible before administration of antibiotic and inoculated one or two drops of blood into 5 to 10 ml of EMJH (Ellinghausen, McCullough, Johnson & Harris)[23] semisolid medium containing 5-fluorouracil. Cultures are incubated at 28°C to 32°C and examined weekly by dark field microscopy for upto 6 weeks before being discarded. The growth is visualised as development of one or more distinct discs of turbidity known as dinger's rings at varying depths in the column of medium[117].

Polymerase Chain Reaction (PCR)

A real time quantitative PCR using Taq Man Chemicals to detect leptospires in clinical samples has been reported[103]. PCR is a method which involves in vitro enzymatic amplification of target DNA sequences, through a series of polymerisations carried out by a thermostable DNA polymerase, primed by a pair of short DNA fragments (Primer) which binds specifically to the sequence of interest. Amplified DNA fragments produced like this can be visualized on ethidium bromide stained agrose gel on an UV transilluminator. PCR has been used to distinguish pathogenic from non-pathogenic serovars[33,86,127]. A fluorescent probe 5'exonuclease PCR assay was described for rapid detection of pathogenic leptospires[128].

Serology

Most cases are diagnosed by serological method. Anbibodies are detectable in the blood approximately 5-7 days after the onset of symptoms. Serological methods can be divided into two groups: genus specific and serogroup specific.

Microscopic agglutination test (MAT)

The microscopic agglutination test is the basis of serological diagnosis and classification. MAT is the key stone for a correct diagnosis[126]. Serial dilutions of serum kept in contact with equal volume of a well grown suspension of leptospires at 37°C for 2-3 hours are read microscopically by estimating 50%

agglutination as the end point titre of the reaction mixture[117].

Macroscopic slide agglutination test (MSAT)

Various macroscopic tests performed in tubes or on tiles or slides have been described. The most often used methods are the ones described by Mazzonelli-Maillaux (1974) [67] heat killed Patoc I antigen and by Galton et al. (1958) [29].

In principle, a certain amount of concentrated killed antigen and patient serum are mixed on a slide and allowed to react for a specified period, after which the presence of agglutination is determined by naked eye.

Tests like IFA, latex agglutination test, ELISA, Dipstick immunoassay also can be done to detect antibodies[34,96,114.].

Animal inoculation

Isolation of leptospires can also be done by intraperitoneal inoculation of laboratory animals like guinea pigs and hamsters. The organisms can be demonstrated from the heart blood and peritoneal cavity after 3-7 days. This technique is not recommended for routine diagnosis and is only useful for isolating organisms from contaminated material[26, 117.].

TUBERCULOSIS

World wide incidence of TB in 2003 was estimated as 8.8 million new cases of which 3.9 million were smear positive and 674 thousands were co-infected with HIV. An estimated 1.7 million people died from TB in 2003 - including those coinfected with HIV[5]. Koch in 1882 isolated the mammalian tubercle bacillus and proved its causative role in tuberculosis by satisfying Koch's postulates. While the majority of people suffering from TB are never diagnosed at all, still less number get correctly treated. It was

estimated that in 1990 there were 7.6 million new cases in developing countries and 400,000 new cases in industrialized countries for a world wide total 8 million new cases[55].

About 5 million new cases were estimated to occur annually in Asia. The current rate in 1994 was 229 per 100,000 persons[104]. Seventy five to 80% of adults in the developing countries have M. Tuberculosis infection. It is also estimated that TB caused 2.9 million deaths world wide in 1990, and all but 4,00,000 of these occurred in developing countries [76].

In developing countries the combination of a high frequency of HIV infection with a high frequency of M. tuberculosis infection results in a high rate of tuberculosis disease as a complication of AIDS. At the same time drug resistant tuberculosis is a growing threat world wide. Incomplete or inappropriate treatment of the disease has spawned the development of strains that are resistant to drugs, that once destroyed the bacteria in 100% of cases[11].

The main factors contributing to the increase are 1. Deterioration of the infrastructure of the health care system. 2. Immigration from countries where tuberculosis is common. 3. Transmission of infection in congregate siblings. Tuberculosis occurs primarily in the age group of 24-45 years. In India infections accounts for 50% cases of FUO and 25% of these are due to tuberculosis[11]. It is difficult to diagnose because of wide range of atypical manifestations of this disease, lack of availability of proper culture and staining facilities in many areas and also non-availability of other rapid diagnostic test like PCR even at tertiary care centers. Sputum positivity for AFB is considered to be the Gold standard though radiological studies also has a diagnostic value.[7]

An effective vaccine that would kill dormant tubercle bacilli would be the real solution to eradicate the disease. Even if there are much better methods of treatment, a huge problem exists in case finding which has not improved with the Directly Observed Therapy (DOTS) expansion programme. However, whatever improvement are made in diagnostic methods, failure to take the first step of investigating patients with symptoms suspicious of tuberculosis is still the most important reason for low rates of diagnosis [74].

Patients usually present with constitutional features like fever, night sweat, weight loss and local features related to the site of illness which are similar in adults and children. Definitive diagnosis of

extra pulmonary TB is difficult. Tuberculous meningitis and TB peritonitis are also present in extra pulmonary TB[42].

DIAGNOSIS

Mycobacterial infection can be confirmed by direct microscopy of samples (Sputum, Cerebro spinal fluid (CSF), Brancho alveolar lavage (BAL), Gastric wash, pleural fluid, pericardial fluid, ascitic fluid and tissue biopsy) by Ziehl-Neelsen or auramine staining and culture. Confirmation of isolates is obtained through standard culture methods and molecular DNA technology[16].

AFB Microscopy

A presumptive diagnosis is commonly based on the finding of AFB on microscopic examination of specimen. The more traditional method-light microscopy of specimens stained with Kinyoun's (or) Ziehl-Neelsen basic fuchsin dyes are satisfactory, although more time-consuming. In suspected pulmonary tuberculosis, three sputum specimens, preferably collected early in the morning are examined.[63]

Mycobacterial culture

Egg or agar-based medium (Lowenstein-Jensen or Middlebrook 7H10) are used for the cultivation of mycobacterium tuberculosis. Isolates are identified on the basis of growth time and colony pigmentation and morphology. A variety of biochemical tests have traditionally been used to speciate mycobacterial isolates[60].

Molecular Techniques

Several test systems based on amplification of mycobacterial nucleic acid are available. These system permit the diagnosis of tuberculosis in as little as several hours, but are costly[63].

Radiographic procedure

Diagnosis is not difficult in a high-risk patient with typical symptoms. A classic chest radiograph shows upper lobe infiltrates with cavities. Immuno- suppressed patients with HIV infection may have "atypical" findings like lower zone infiltrates without cavity formation[63].

BRUCELLOSIS

Brucellosis is an important zoonotic disease. It is a global problem, which remains in the background for lack of awareness. Mildness and chronicity of the disease makes the diagnosis difficult.

Brucellosis is an occupational hazard. It is primarily a disease of animals transmitted directly or indirectly to man. Dairy workers, shepherds, veterinarians, abattoir workers and animal husbandry personnels are particularly at risk. It constitutes an uncontrolled public health problem in many developing countries[75]. There are many reports on the incidence of brucellosis in India. In a study conducted by Mathur et al[66] , the incidence of brucellosis among animal handlers was reported to be 8.5%.
Acute brucellosis in its more severe forms sometimes causes characteristic intermittent waves of temperature that gave the name "undulant fever" to the human disease[113]. Brucellosis is an acute febrile zoonotic disease transmitted to dogs, birds and humans. The diagnosis of *Brucella melitensis* and *Brucella abortus* infection in human can be done by Brucella agglutination assay and dipstick assay[31]. Reports of human cases of brucellosis are still few in number and many are based only on serological test, not on isolation of the organism[32].

DIAGNOSIS

The diagnosis of brucellosis based exclusively on Brucella isolation presents several drawbacks. The slow growth of brucella in primocultures may delay diagnosis for more than 7 days. Also blood culture sensitivity is often low, ranging from 50 to 90%. Hence, serological tests play a major role in cases when the disease can't be detected by blood culture. The most commonly used tests are the serum agglutination test (SAT), the Coomb's anti-Brucella test, the Rose Bengal test and complement fixation. Brucella capt, a new serological test for the diagnosis of human brucellosis based on immunocapture - agglutination of total anti-Brucella antibodies was studied and evaluated by Antonio Orduna. Brucella capt and Coombs titers are always high, whether SAT titers are higher or lower than 1:160.[4] PCR also can be done to detect infections in these cases[72].

PNEUMONIA

Pneumonia is defined as acute respiratory illness, can be broadly categorized as community acquired, hospital acquired or those occuring in the immuno- compromised host or damaged lung (including suppurative and aspirational pneumonia). Community acquired pneumonia is usually spread by droplet inhalation. Hospital acquired pneumonia usually occurs at least 2 days after admission to hospital. Suppurative pneumonia produced by infection of previously healthy lung tissue with *Staph. aureus* or *Klebsiella pneumoniae*. Possible routes include aspiration, aerosolization, hematogenous spread, and direct spread from a contiguous infected site [59].

Commonest bacteria causing different type of penumonia[116]

Community-acquired pneumonia	*Hospital-acquired pneumonia*	*Suppurative pneumonia*
Strep. penumoniae *Chlamydia pneumoniae* *Mycoplasma pneumoniae* *Legionella pneumoniae* *Staph. aureus*	*E. coli* *Pseudomonas* *Klebsiella Sp.* *Staph. aureus*	*Staph. aureus* *Klebsiella pneumoniae* *Strep. pneumoniae* *Staph. aureus* *Strep. pyogens* *H. influenzae*

DIAGNOSIS

Diagnosis can be made by direct gram stain and culture of the sputum. Blood culture also can be done to find out hematogenous infection. Radiological finding may be typical or vary from local consolidation to multiple patchy infiltrates and abscess formation may or may not be related[6].

BACTEREMIA

Bacteremia may be defined as a condition of simple presence of bacteria in blood without any multiplication which is often silent and transient. Sources of bacteremia are determined according to Centre for Disease Control and Prevention, definitions of nosocomial infections and the presence of clinical signs with isolation of organism from the presumed source[95].

Infection Sources[95]

Route of infection	*Percentage*
Endovascular location	
- Heart valve	7.4%
- Other	5.1%
Soft tissue	13.5%
Bone	11.9%
Urinary tract	2.9%
Respiratory tract	5.4%
Miscellaneous	2.6%
Unknown	21.8%

Commonest bacteria isolated from blood[2]

Microorganism	**Percentage**

E. coli	36.1%
Streptococcus sp.	19.4%
Salmonella sp.	13.8%
Enterobacter sp.	11.1%
Staphylococcus aureus	11.1%
Aeromonas hydrophila	2.8%
Klebsiella penumoniae	2.8%
Proteus mirabilis	2.8%
Burkholderia pseudomallei	2.8%

A significant clinical complication of *K. penumoniae* infection is peripheral blood dissemination, which results in bacteremia coincident with the localized pulmonary infection[129]. The inability to clear blood borne bacteria can lead to a state of over whelming bacteremia, which can culminate in multiple - organ dysfunction syndrome and an increased mortality rate[115].

DIAGNOSIS

Diagnosis can be made by blood culture for isolation of organism. Several methods for rapid detection of pathogens in human blood have been described and most of them have used PCR or fluorescently labeled probes. A simple and direct whole-cell hybridization assay with species-and genus-specific, fluorescently labelled oligonucleotide probe was developed. Oligonucleotide probes, fluorescently labeled with fluorescein isothiocyanate, directed against the variable regions of the 16S rRNAs of the bacterial species and/or genera were used[43].

RICKETTSIAL DISEASES

H.T. Ricketts, an American Physician, first recognised the causative agent of typhus and spotted fever in 1916.

The family Rickettsiaceae includes a diverse group of organisms that share common features such as intracellular growth and transmission by arthropod vectors. Rickettsiae normally enter the body through the bite or faeces of an infected arthropod vector. Initial symptoms of the disease are headache, fever often accompanied by myalgia, anorexia, vomiting, abdominal pain, diarrhoea, photophobia and cough. Timely

and accurate diagnosis of Rickettsial disease followed by administration of an appropriate antibiotic may mean the difference between the death of the patient and uneventful recovery[17].

The lack of widely available reliable diagnostic tests that can detect the diseases in their early stages remains a problem, particularly when symptoms are non-specific. The oldest and most widely used laboratory method was the Weil-Felix test, which relies on agglutination of the somatic antigen of *Proteus sp*[105].

The Rickettsial diseases are divided into four main groups.

1. Typhus fever	2. Spotted fever
3. Scrub typhus	4. Q fever

Epidemic typus is caused by Rickettsia prowazeki. Transmission is by human body louse. The organism multiplies in the epithelial cells of the louse gut and passes out in the faeces. Man is the only other participant in the cycle and so the disease can be controlled by human delousing[110].

Endemic typhus and Murine typhus pertaining to rats and mice. It is caused by Rickettsia typhi. The disease occurs in rats and spread by the rat flea *Xenopsylla cheopis*[1].

DIAGNOSIS

Diagnosis is made by the serological test like Weil-Felix reaction by using non-motile strains of *Proteus vulgaris* OX19 and OX2 and motile strains of *Proteus mirabilis* OXK antigens. Species-specific antibodies may be detected by complement fixation, microagglutination and fluorescence in specialised laboratories[17].

URINARY TRACT INFECTION (UTI)

Urinary tract infection is defined as bacteriuria i.e. the multiplication of the organism in the urinary tract

and the presence of more than a hundred thousand (10^5) per ml in the midstream sample of urine (MSU). Fever is a very common symptom seen in upper urinary tract infection. UTI may be symptomatic or asymptomatic. Infection occurs in 1 to 3% of school girls and then increasing markedly in adolescence with the onset of sexual activity in adolescence. UTI may be complicated or umcomplicated. Complicated infections may result in permanent renal damage where as uncomplicated infections rarely do so and are caused by a single strain of organism[9].

Causes of bacterial agents in urinary tract infection are depicted below[9]

Organism	Uncomplicated	Complicated
E. coli	80%	50%
Proteus sp.	8%	12-15%
Klebsiella sp.	8%	12-20%
Staph. saprophyticus	0-1%	1-2%
Staph. epidermidis	0-1%	1-2%
Enterococci	0-1%	4-5%
Enterobacter	< 1%	< 1%
Citrobacter sp.	< 1%	< 1%
Acinetobacter sp.	< 1%	< 1%
Ps.aeruginosa	0-1%	8-12%
Staph. aureus	0%	< 1%

Virulence characteristics of uropathogenic *E. coli* causing urinary tract infection were studied in different group of peoples like antenatal, postnatal, urology patients and rehabilitation group at CMC, Vellore.[89] There are two routes of infection causing UTI, ascending route from perianal region and haematogenous route by bacteremia[9]

DIAGNOSIS

Diagnosis can be made by direct examination of urine, gram stained from uncentrifuged urine and culture by semiquantitative method with antimicrobial suscpetibility test.

Direct microscopy examination can be done with centrifuged deposit of urine for detection of pus cells, epithelial cells, cast and bacteria.

Culture can be done by semiquantitative method and organisms are identified based on biochemical reactions. Another method commercially available is plastic slides coated with CLED (cysteine lactose electrolyte deficient) agar on one side and MacConkey's agar on the other side. The slide is dipped in urine and placed in sterile container and incubated. Viable count is obtained by comparing with manufacturer's chart[9]. Dipstick test for nitrate is used as a surrogate marker for bacteriuria. It should be noted not all

uropathogens reduce nitrates to nitrite i.e. *enterococci*, *S. saprophyticus* and *Acinetobacter sp.* do not and give false negative results[22].

MALARIA

Malaria has always been a major public health concern, probably representing the most important parasitic disease in humans. It has been infecting human kind for millennia. Malaria presents a diagnostic challenge to laboratories in most countries. After the initial success, India is now unable to get the upper hand in malaria control and over the past decade or so, the number of malaria cases has remained unchanged with the addition of increasing Falciparum malaria. The problem is essentially due to man-made environmental alteration which provides excellent opportunities for the mosquito vector to breed. The new paradigm of malaria consists of new eco-types such as irrigation malaria, urban malaria, development project malaria, migration malaria, and border malaria[100].

Malaria is the most important of the parasitic diseases, affecting more than 1 billion people and causing between 1 and 3 million deaths each year[80].

The clinical features include several days of continuous fever and gradually spleen and liver may enlarge. The typical attack comprises of three distinct stages namely 1. Cold stage 2. Hot stage 3. Sweating stage. These are followed by an afebrile period in which the patient feels greatly relieved. The classical three stages may not always be observed due to maturation of generations of parasites at different times. Periods of latency may last for several weeks or months. Sometimes *Plasmodium falciparum* parasites can be sequestered and are not present in peripheral blood. Thus a *Plasmodium falciparum* infection could be missed due to absence of the parasites in a blood film. Majority of malaria cases occur in rural areas where there is little or no access to reference laboratories and in many areas microscopy is not available.

DIAGNOSIS

Blood smear

Thin blood smear can be stained with 0.15% Leishman stain or Jaswant Singh-Bhattacharya stain (JSB stain) and thick blood smear can be stained with JSB stain after dehaemoglobinization.

A study was done to compare microscopic examination of blood film with newly developed simple dipstick antigen-capture assay which detects the presence of Parasite Lactate DeHydrogenase (PLDH) antigen of malarial parasite in lysed whole blood sample. The PLDH first binds to a gold labeled antibody particle. This complex then migrates up the test strip where it is captured by an immobilized second antibody. A visual antibody-antigen-antibody complex is then formed at the reaction site[12].

Conventional pheripheral blood smear examination remains the gold standard for diagnosis of malaria in malaria endemic countries. However the technique is labour intensive and time consuming and may give poor results in cases with low parasitaemia. In recent years, numerous quick and new techniques for diagnosing malaria have been developed, one such being direct AO (acridine orange) staining technique

which is 93.3% sensitive and 99.25% specific[78].

Demonstration of circulating malarial antigen may be done by gel-diffusion and counter-immuno electrophoresis[40].

Demonstration of parasites in blood by thick blood smear, thin blood smear, Quantitative Buffy Coat (QBC) and Acridine orange staining by using fluorescent microscopy are done. Under serological method antibodies against malarial parasite can be demonstrated in serum by using Indirect haemagglutination (IHA), Indirect fluorescent antibody (IFA) and Enzyme-linked immunosorbent assay (ELISA). Enzyme immuno assays like Dipstick, DOT ELISA are used to detect *Plasmodium falciparum* histidine rich protein - 2 antigen. PLDH test used to detect the parasitic enzyme LDH which is produced by *Plasmodium species*. PCR is done to detect the specific nucleic acid sequences[106].

FILARIASIS

Filariasis is a major public health problem in India. With the continuous change in environmental factors, urbanization and availability of newer diagnostic tools, the estimation of 40% global burden due to filariasis in India is found to increase. Recent estimates show that there are approximately 119 millions persons affected with this disease world wide: 106.19 and 12.91 millions with Bancroftian and Brugian filariasis respectively. In India currently 411.65 million people are exposed to the risk of infections, of which 46 million are protected, and there are 31.26 million micro filaria carriers and 48.11 million persons with chronic disease. About 95% of the total infection is due to W. bancrofti. The disease due to its prolonged course and non-fatal nature has attracted little attention of the health planners, thus a low priority has been accorded by the states to the National filaria control programme. [71]

The clinical manifestations of lymphatic filariasis tend to vary in different geographical locations. Depending upon the infection status of the individuals in endemic areas and the resulting clinical consequences there of, five main categories of the filarial clinical spectrum have been recognised[125]. They are 1. asymptomatic amicrofilaraemia, 2. asymptomatic microfilariaemia 3. acute manifestations including fever, chills, malaise, headache, nausea and vomiting[124]. 4. Chronic manifestations like lymphatic obstruction and fibrosis of male genitals and limbs of both sexes[84,85]. 5. allergic manifestations.

DIAGNOSIS

Detection of the parasite in peripheral smear and ultrasound lymphangio scintigraphy are the techniques commonly employed. Estimation of circulating filarial antigen (CFA) by Og4C3 ELISA can also be done[14].

BABESIOSIS

Babesiosis is a disease which occurs in splenectomised and immunosupressed persons and transmitted by

arthropod vectors usually Ixotid ticks. *Babesia microti* was reported to cause infection in a patient with intact spleen in the year 1969[106]. The disease in such individuals is usually self limiting, so that specific treatment is not required.

DIAGNOSIS

Diagnosis of babesiosis depends on the demonstration of the intra erythrocytic parasites in thin blood smear by microscopy and inoculation of blood into susceptible animals. Indirect fluorescent antibody (IFA) test using intra erythrocytic parasites as antigens is a useful serological test in the diagnosis of *B. microti* [30,123]

LEISHMANIASIS

This disease is prevalent in various parts of the world, involving reticulo-endothelial system resulting in visceral, cutaneous and mucocutaneous leishmaniasis. Kala-azar is the most serious form of leishmaniasis, which is a life threatening disease.

DIAGNOSIS

Diagnosis can be made by demonstration of the parasite on stained thin blood smear (Leishman stain) by microscopy. Cultured parasites may be speciated by PCR. Serological tests like CFT, IFA, IHA are done to demonstrate the antibodies in the blood of visceral leishmaniasis[15,57].

TOXOPLASMOSIS

Toxoplasmosis is an important cause of still birth and congenital abnormality. Congenital toxoplasmosis occurs in patients suffering from AIDS. Acute infection of the central nervous system (CNS) results in focal or diffused meningo encephalitis with area of necrosis and in AIDS characteristically necrotising encephalitis. Serological evidence suggest that human infection is common, presumably as a transient febrile illness or a subclinical attack and is of world wide occurrence[20].

DIAGNOSIS

Diagnosis can be made by isolation of *Toxoplasma gondii* and sero diagnosis by latex agglutination test, IHA, CFT and ELISA[28].

AMOEBIASIS

Entamoeba histolytica invades colonic mucosa producing characteristic ulcerative lesion and profuse diarrhoea. Systemic infection may arise leading to abscess formation which is accompanied by fever and other symptoms.

DIAGNOSIS

Diagnosis can be made by stool examination using microscopic examination of wet mounts and permanent stained mounts. The stains used are iron-haematoxylin and Gomoris trichrome stain. Cultures of amoebae are more sensitive but are not routinely available[102].

Serological tests like IHA, IFA & ELISA can be done to detect serum amoebic antibodies in the invasive intestinal amoebiasis. However these tests are of no or little value. Molecular methods like DNA probe have been used recently to identify *E. histolytica* in the stool specimen. Ultra sonogram can also be done for the amoebic liver abscesses[106].

MATERIALS AND METHODS

Study of Place : Institute of Microbiology
Madras Medical College
Govt. General Hospital, Chennai-3.

Study Period : Dec. 2003 to Nov. 2004

STUDY POPULATION

The study included 124 patients, admitted to the Government General Hospital, Chennai with fever of 1 week and more duration and of the age group 12 years and above. Only bacterial and parasitic causes of the FUO infections were included in this study. Immuno deficient patients and patients who were treated already with antibiotics were excluded from this study. Viral and fungal causes of FUO were not investigated in the present study.

MATERIALS

Detailed history was obtained from all the patients and a complete clinical examination was done. Patients were subjected to investigations based on their clinical picture. The data collected from the patients were documented in a proforma.

METHODS

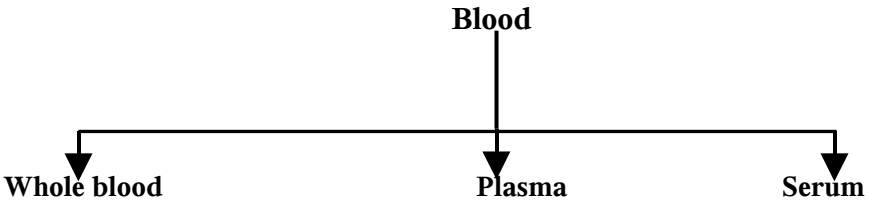
All 124 patients were subjected to the basic investigations irrespective of the provisional diagnosis. Relevant data were collected in the prescribed proforma along with socio-economic details.

BLOOD

Ten ml of blood was collected by vene puncture with aseptic precautions from all 124 patients. The blood was divided into three portions

1. Whole blood
2. Blood with EDTA – plasma
3. Blood in plain test tube – Serum

The blood (Whole blood, plasma, serum) were processed for the diagnosis of the following infections.



Blood Culture
Leptospira Cultivation
Rapid Test for Malaria
DEC provocation test (filariasis)
Blood smear for
-Malarial parasite
-Filarial Worms
-Babesia
-Leishmania
-Toxoplasma gondii

Leptospirosis
- DFM
- Fontana's Staining

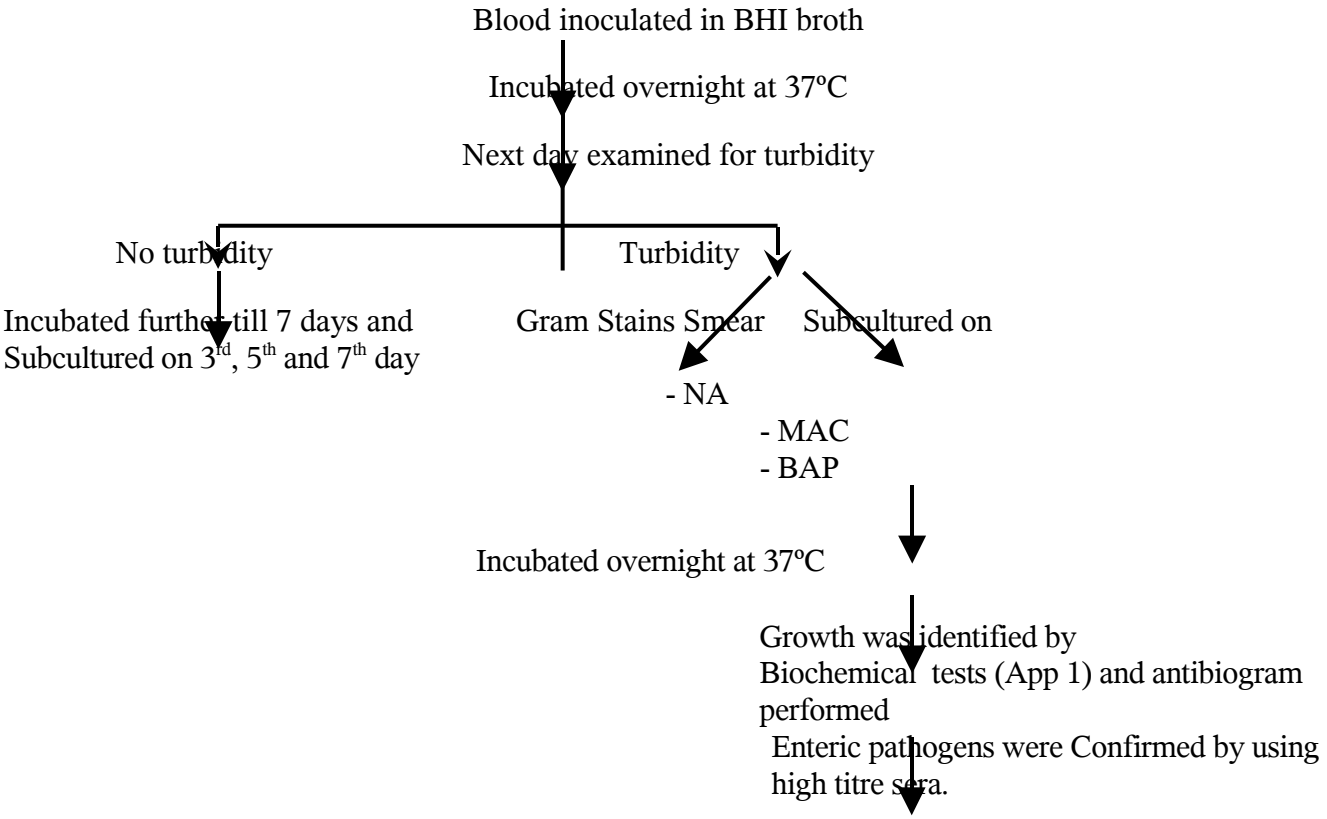
Leptospirosis
-PCR - Ag
-MSAT - Ab
-MAT - Ab

Typhoid
-Widal Agglutination test
-Rapid Slide test for typhoid

Brucellosis
-Brucella Agglutination test

Rickettsial Infection
-Weil-felix test

Blood culture for enteric and non-enteric pathogens



LEPTOSPIROSIS

Examination of Leptospira under Dark Field Microscopy (DFM)

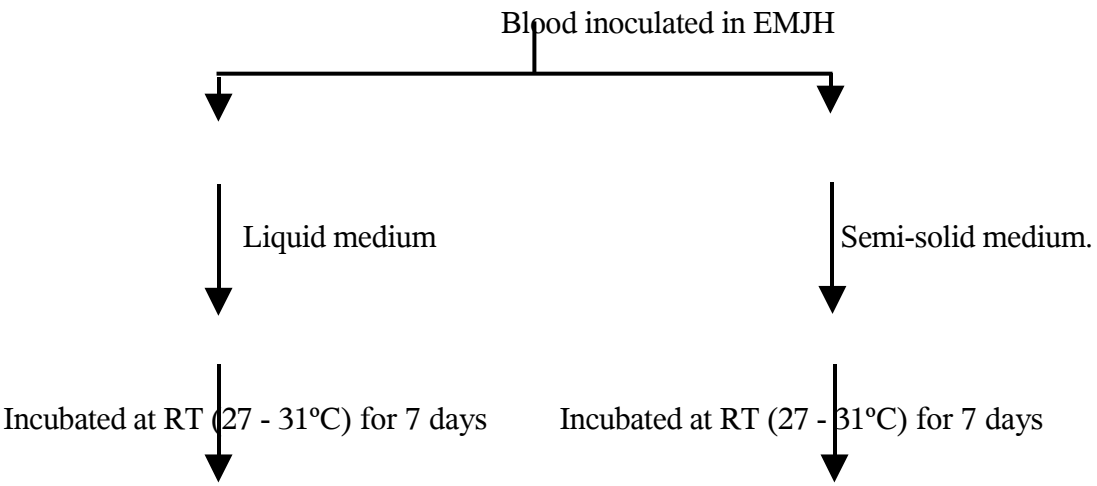
A wet film preparation of the patients plasma was made and seen under DFM for the typical morphology and motility pattern of the leptospires (Appendix - 2).

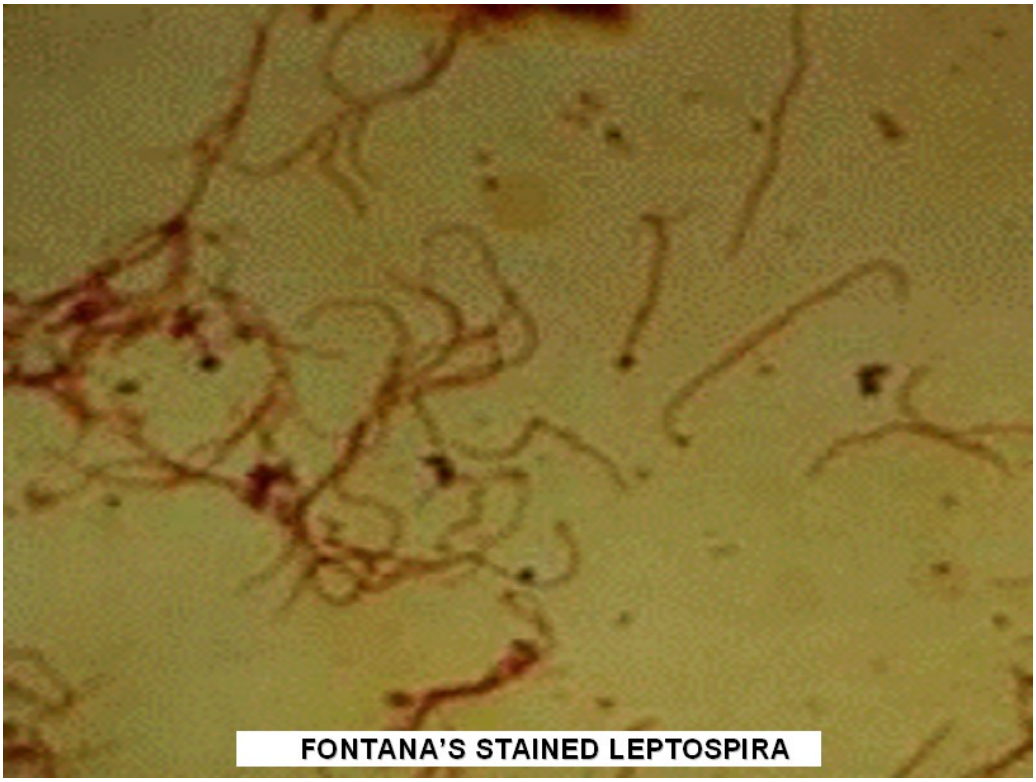
Fontana's silver impregnation staining of leptospires was done on smear from plasma (Appendix - 3). Leptospires appears as brownish black against yellowish brown background.

Cultivation of leptospira

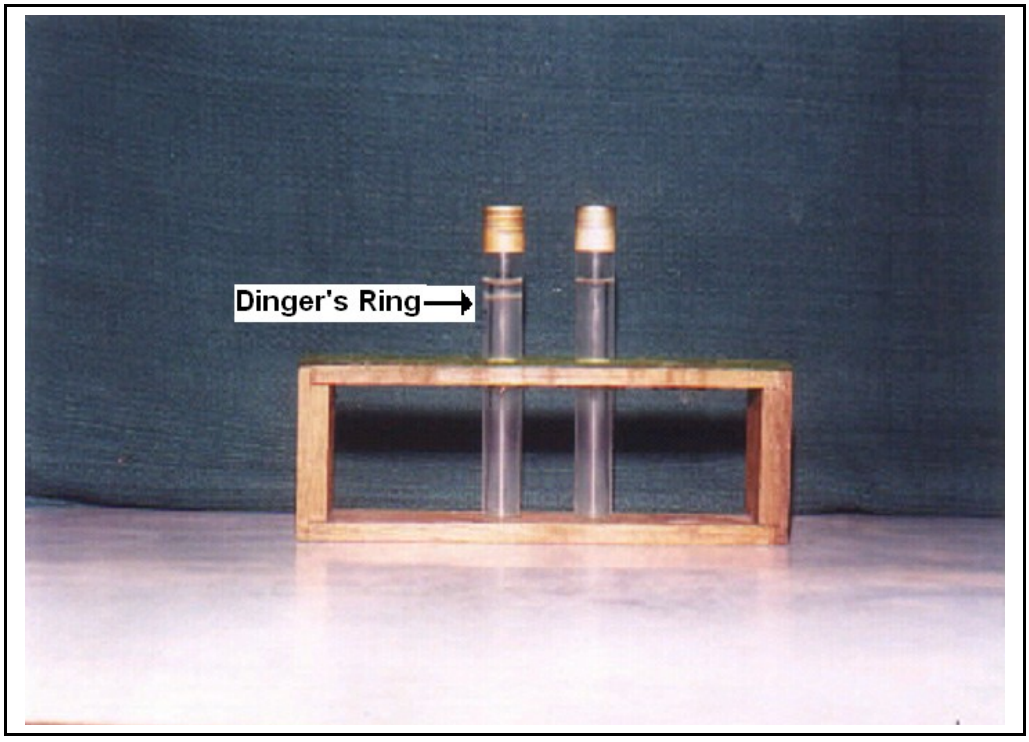
Few drops of blood samples were inoculated into the tubes containing 5 ml of EMJH[23](Ellinghausen-

McCullough-Johnson-Harris) liquid and semisolid media (Appendix 4).





CULTIVATION OF LEPTOSPIRA



POLYMERASE CHAIN REACTION (PCR)

The PCR involves the enzymatic amplification of DNA *in vitro*. This method is capable of increasing the amount of the target DNA sequence in a sample by synthesizing many copies of DNA segment. PCR was carried out as per methodology of Merien et al (1992)[69]. Primers used in this procedure were G1&G2[33].

G1 - 5'- CTG AAT CGC TGT ATA AAA GT-3'

G2 - 5'- GGA AAA CAA ATG GTC GGA AG-3'

PCR products were analysed by gel electrophoresis[119]**.** If positive an amplified single PCR product at 285 base pair band is obtained (Appendix - 5).

MALARIA, FILARIASIS, BABESIOSIS, LEISHMANIASIS, TAXOPLASMOSIS.

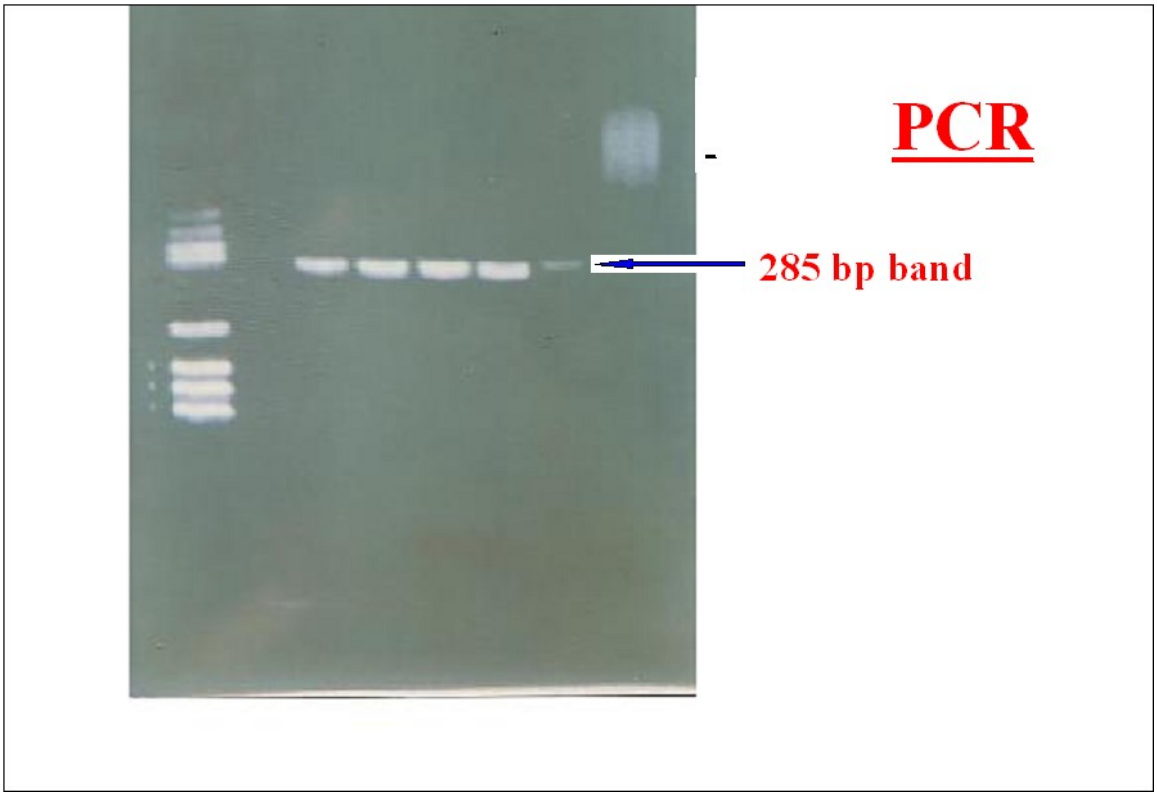
Peripheral Blood Smear

A drop of blood was used for preparing blood films. Leishman stain (0.15%) was used for staining the smear (Appendix-6,7).

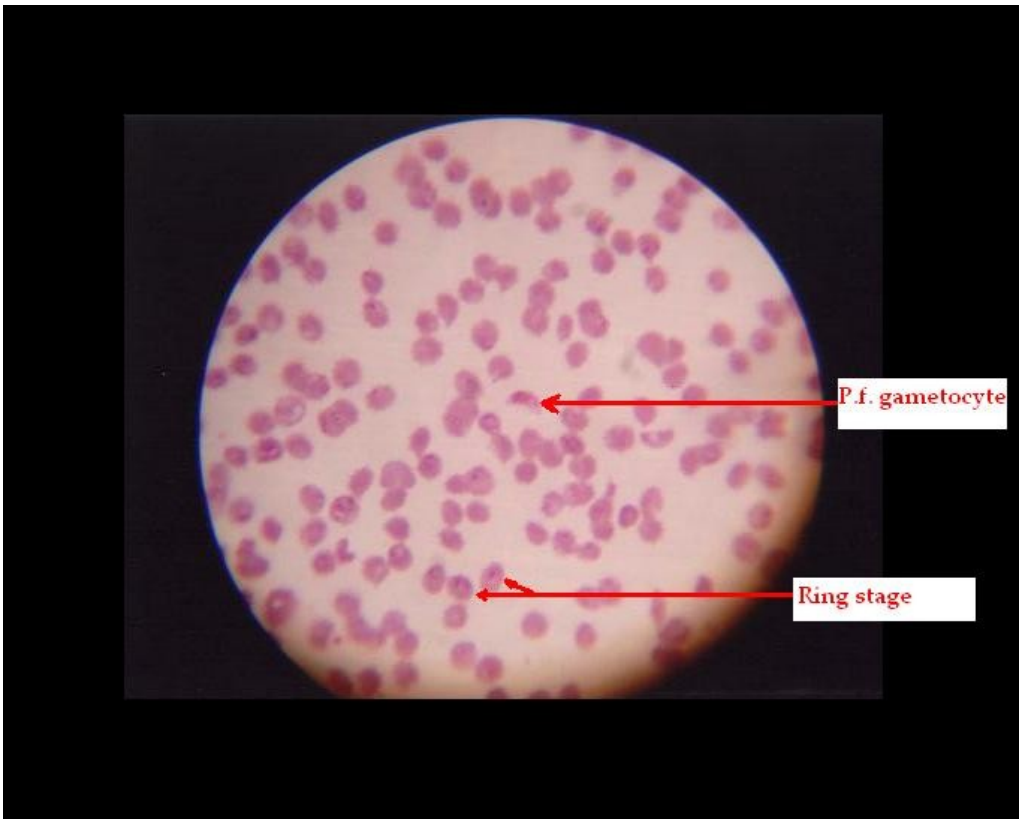
Both thick and thin smears were done to detect malarial parasite and to identify the species respectively. The thick smears were also examined for *Microfilaria, Babesia,* amastigote forms of *Leishmania* and *Toxoplasma gondii*. Minimum 100 fields were examined.

In suspected cases of filariasis DEC provocation test was done (Appendix-8).

POLYMEREASE CHAIN REACTION



BLOOD SMEAR - LEISHMAN STAIN - MALARIAL PARASITE



RAPID MALARIA TEST

A highly specific and sensitive test was done to detect the presence of Plasmodium Lactate DeHydrogenase (PLDH) an enzyme produced by all forms of the malarial parasites. The presence of PLDH is revealed by using monoclonal antibodies directed against isoforms of the enzyme. There is no cross reaction with human LDH enzyme (Appendix - 9).

INTERPRETATION OF RESULTS[101]

1. Positive

P. falciparum : one control band plus two test bands.

P. vivax : one control band plus one test band

2. Negative

One control band at the top of the test strip.

SEROLOGICAL TESTS

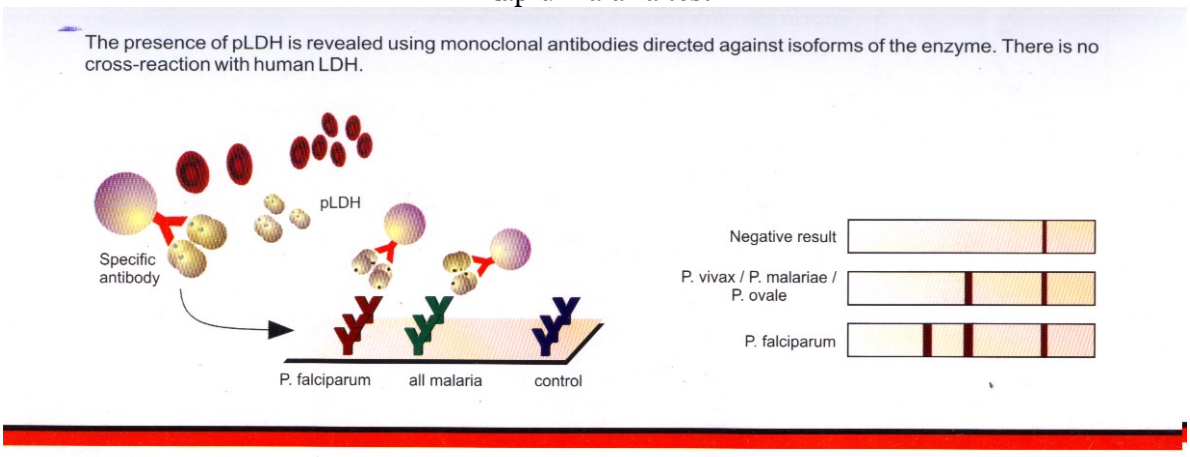
In case of a positive serological test the patient was asked to come after 10 days to find out the rise in titre.

LEPTOSPIROSIS

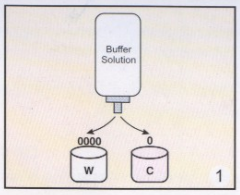
Macroscopic Slide Agglutination Test (MSAT) (Appendix - 10)

This test for the detection of antibodies against the leptospires was performed by using pooled of leptospiral killed antigens.

Rapid malaria test

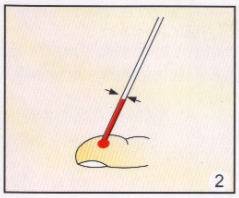


TEST PROCEDURE

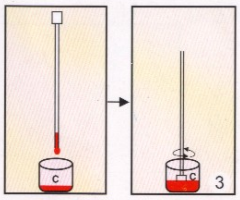


Dispense 1 drop of buffer into the conjugate well and 4 drops into the wash well.

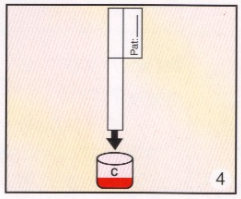
Wait for 1 minute.



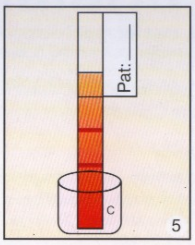
Take one drop of blood (~ 10mL).



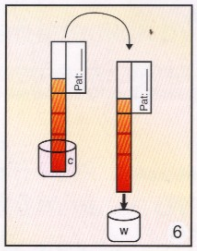
Add one drop of blood to the conjugate well and mix.



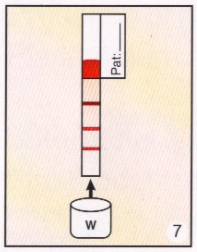
Place the dipstick vertically into the conjugate well and **allow to stand for 10 minutes.**



The blood migrates towards the filter pad and the control band will appear progressively.



Transfer the dipstick from the conjugate well to the wash well and leave it until the reaction field of the dipstick is cleared.



When the control band becomes clearly visible (within 5-10 minutes), then the results can be read.

C Conjugate well (Violet Colour)

W Wash well (Colourless)

RAPID MALARIA TEST - RESULTS

The following leptospiral sero groups were used:

1. Icterohaemorrhagiae *2. Autumnalis*
3. Australis *4. Hebdomadis*

5.Pomona *6. Pactoc*

Interpretation of Results [111]

A positive result was read by examining for the presence of agglutination and graded.

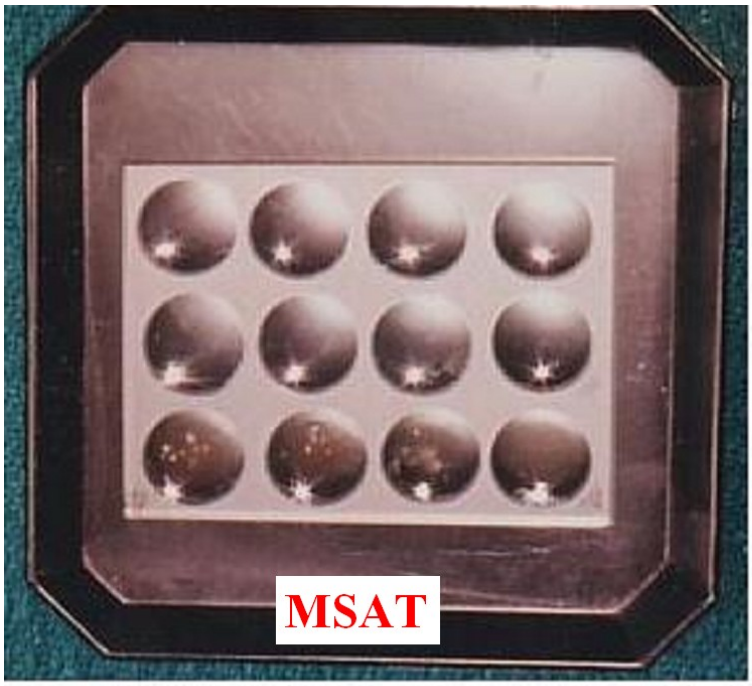
1.	Clumps of agglutination with complete clearing of leptospiral antigen	4+
2.	Obvious agglutination but partial clearing of suspension	3+
3.	50% agglutination	2+
4.	25% agglutination	1+
5.	No agglutination and uniformity of serum antigen mixture	Negative

An agglutination of ≥ 2 considered as positive.

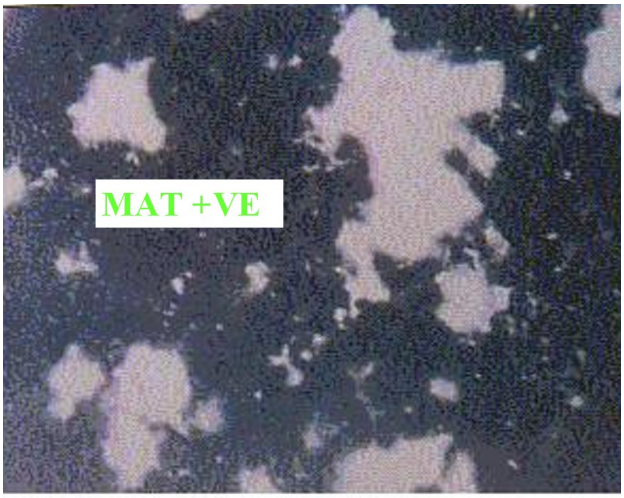
Microscopic Agglutination Test (MAT)

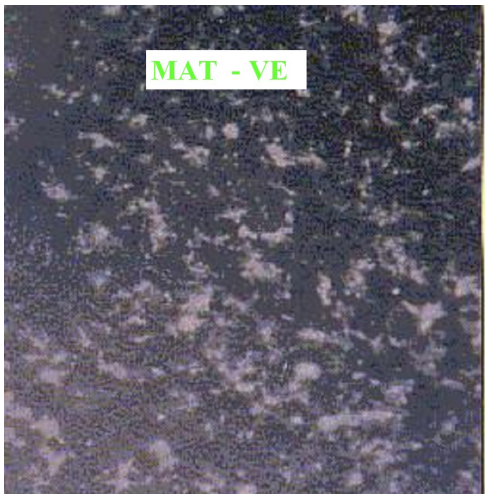
Microscopic agglutination test is the "gold standard test" to detect sero groups of leptospirosis (Appendix - 11). This test was performed by using live antigens of the same sero groups used for MSAT.

MSAT SHOWING +VE & -VE RESULTS



MICROSCOPIC AGGLUTINATION TEST





Interpretation of Results[121]

The MAT titre was expressed as the highest serum dilution that resulted in 50% agglutination of leptospires. A titre 1:80 and above was considered as the significant titre value for positivity of leptospirosis.

TYPHOID

Widal agglutination test

It is an antibody detection tube agglutination test, using bacterial suspension of *S.typhi, S.paratyphi A* and *S. paratyphi B* treated to retain only the somatic (O) or flagellar (H) antigens.(Appendix 12) Antibodies appear in blood by the end of first week.

Rapid slide agglutination test for typhoid.

This is a rapid screening test done for rapid diagnosis of typhoid fever, by identifying the antibodies to

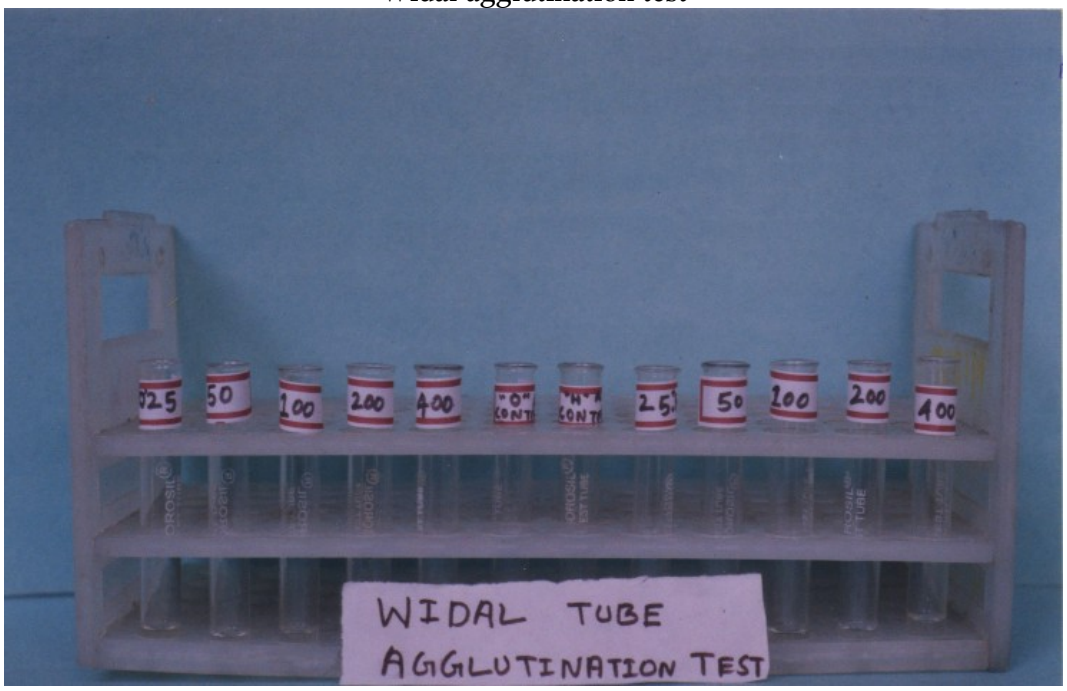
Salmonella typhi, paratyphi A, *paratyphi B* and the results were obtained within 5 minutes(Appendix 13).

The results were compared with specific serological tube agglutination test (Widal).

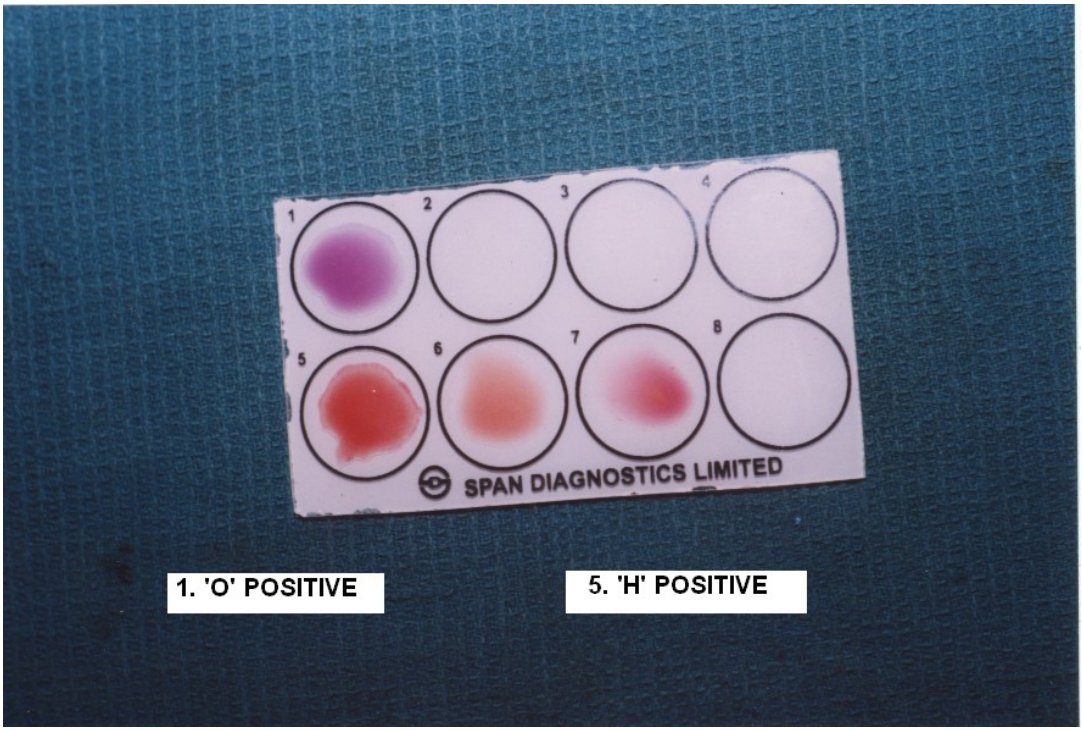
Antigen used for Widal agglutination test

Antigens used	
S. typhi O *S. typhi* H *S. paratyphi* AH *S. Paratyphi* BH	Obtained from King Institute of Preventive Medicine, (KIPM) Guindy

Widal agglutination test



RAPID SLIDE AGGLUTINATION TEST - TYPHOID



Interpretation of Results

- `H' agglutination seen as formation of loose, cotton wooly clumps.
- `O' agglutination seen as granular deposit at the bottom of the tube.
- Controls with no agglutination seen as a compact button of settled organisms.

The criteria for diagnosis were :

`O' agglutination titre of $\geq$ 1 in 100

`H' agglutination titre of $\geq$ 1 in 200

BRUCELLOSIS

Brucella agglutination test

Agglutinins detected in the patients serum by standard agglutination test were usually either IgM or IgG.

False negative results due to prozone phenomenon can be avoided by testing a series of two-fold dilutions of inactivated serum from 1 in 20 to 1 in 640 in 0.4% phenol saline (Appendix - 14).

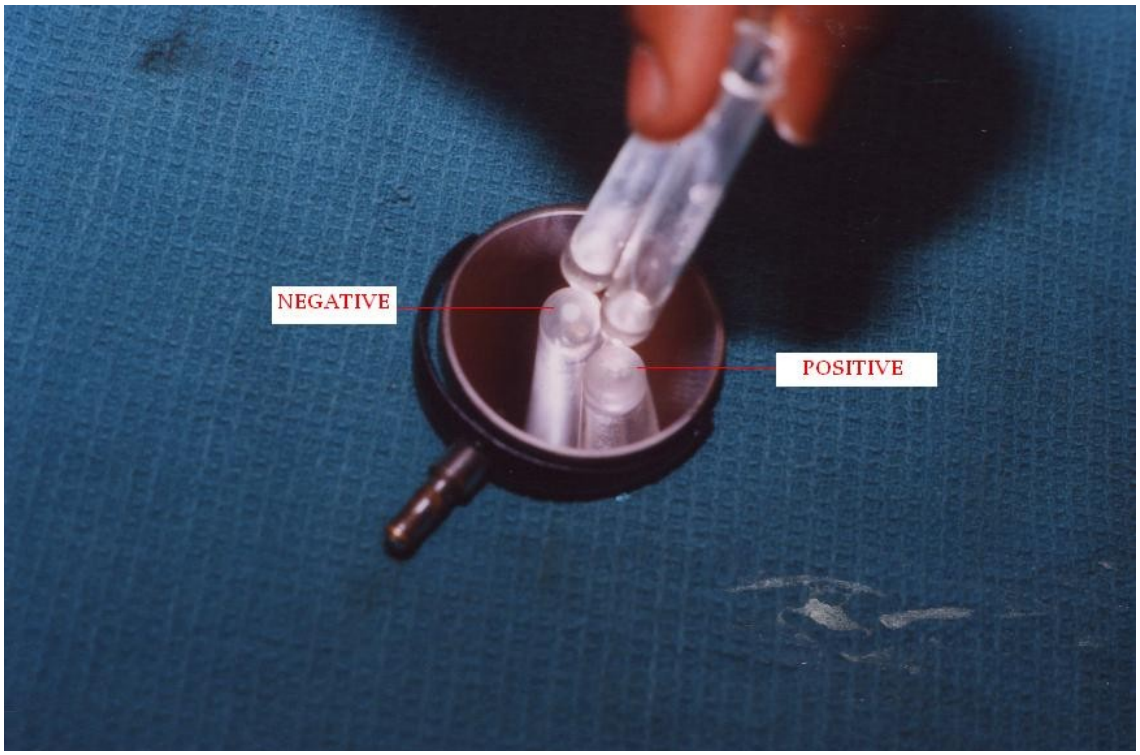
Antigens used

Br. Melitensis obtained from KIPM - Guindy
Br. abortus

Interpretation of Results

Partial or complete clearing with agglutination reaction visible by naked eye (after gentle agitation of the deposit) reported as positive.

INTERPRETATION OF WIDAL AGGLUTINATION TEST - RESULT



1 in 80 and above is the significant titre for positivity.

RICKETTSIAL INFECTIONS

WEIL-FELIX TEST

This test depends on a fortuitous similarity of certain carbohydrate antigenic determinants which occur in most species of pathogenic *Rickettsiae* and in the OX19, OX2, motile strains of *Proteus vulgaris* and OXK non-motile strains of *Proteus mirabilis*[60]. The antibody titration may also be run as a tube test, in which complete agglutination is indicated by complete clearing of the supernatant fluid and the formation of white flocculent masses in the bottom of the tube.(Appendix 15)

ANTIGENS

Proteus OX19
Proteus OX2 } Obtained from KIPM - Guindy
Proteus OXK

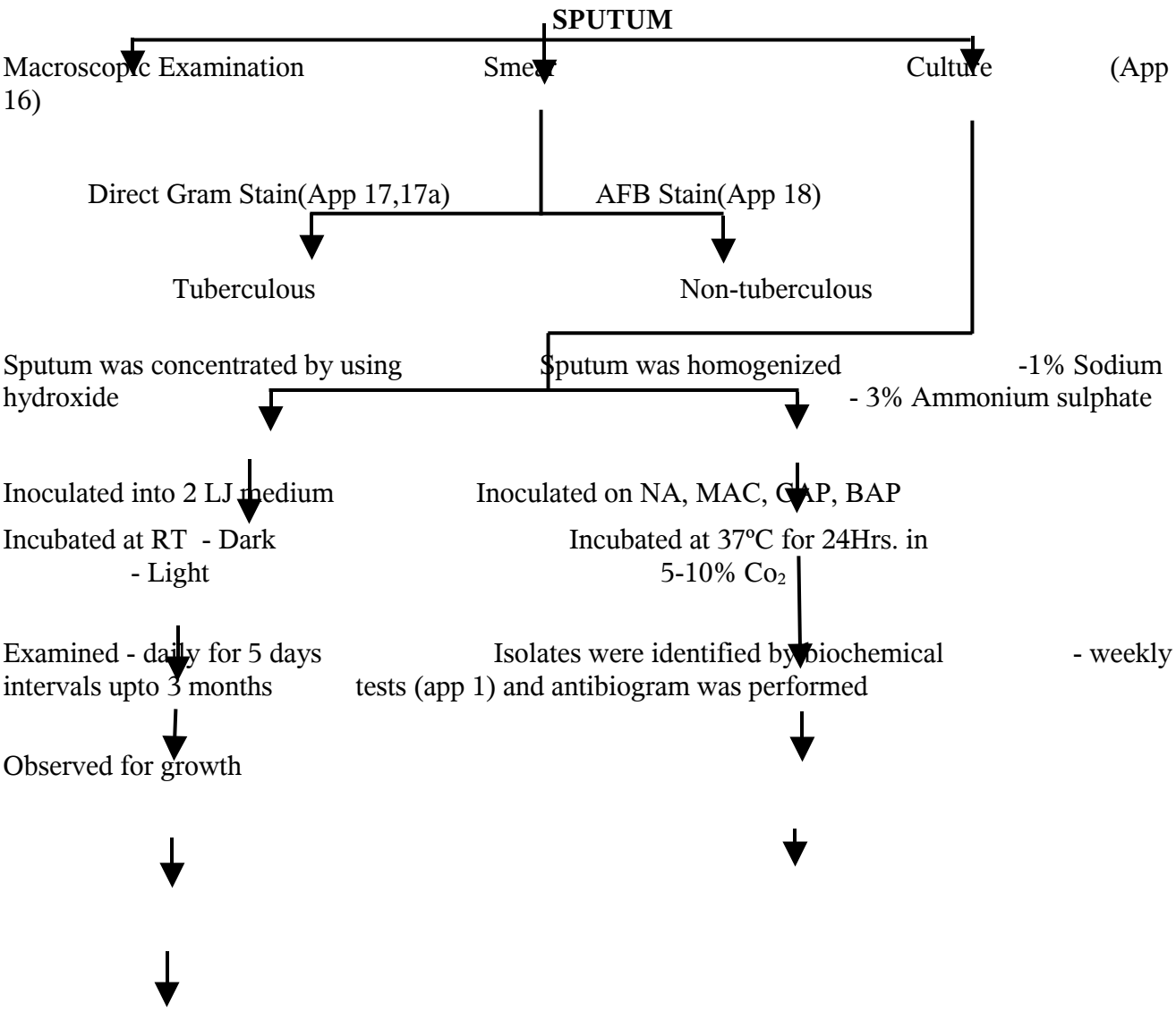
Positive control sera were obtained from Christian Medical College (CMC), Vellore.

Interpretation of Results

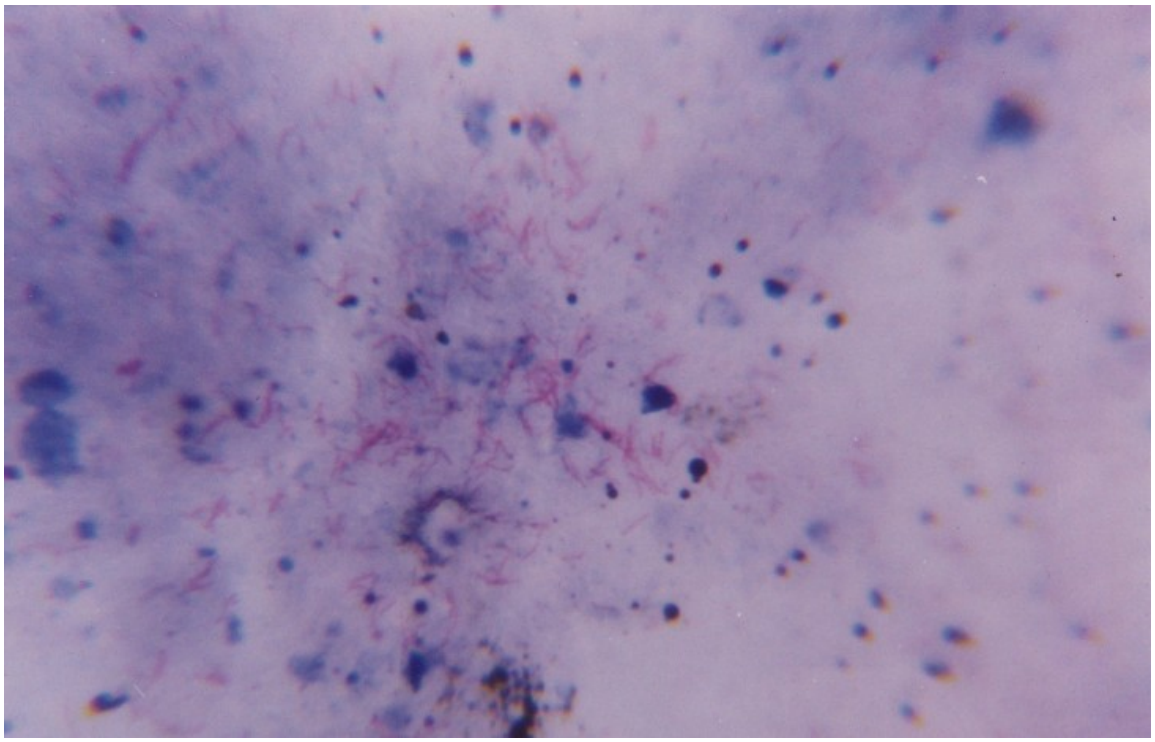
A serum titre of 1:80 and above is considered significant, but four-fold increase of antibody considered diagnostic.

RESPIRATORY TRACT INFECTION (SPUTUM)

Patients were instructed to have a mouth wash and gargle well with sterile distilled water and then asked to cough into a sterile wide mouth bottle and the sputum was immediately processed.

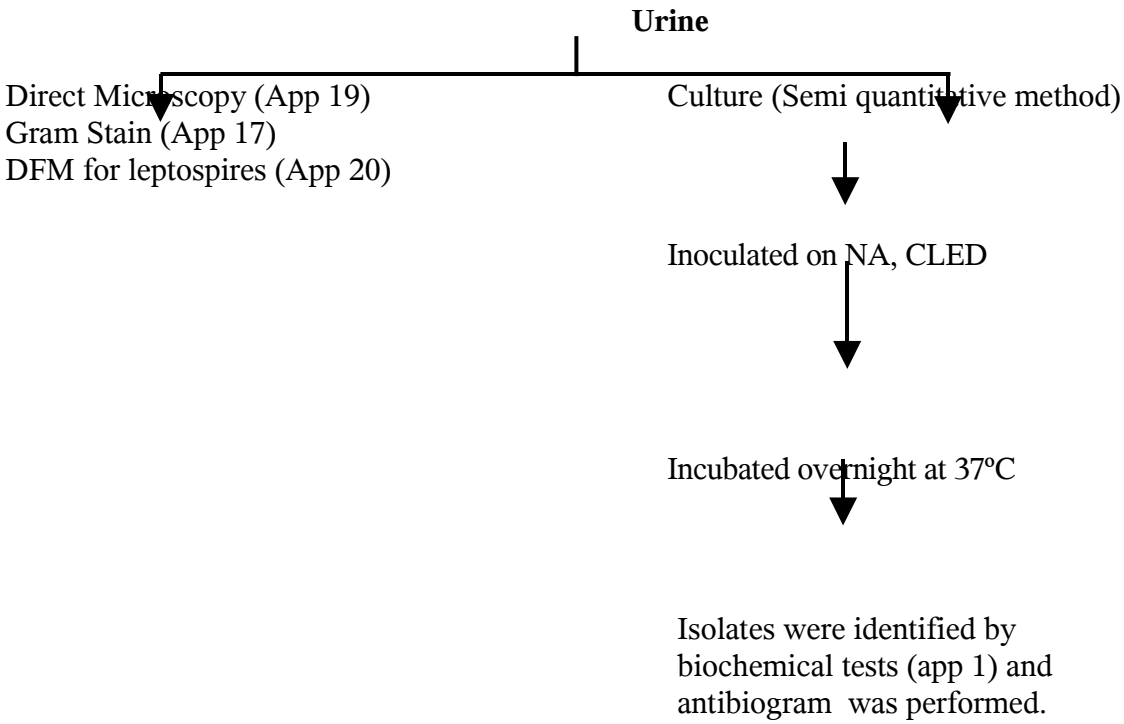


AFB - SMEAR



URINARY TRACT INFECTION (URINE)

Freshly voided CLEAN-CATCH MIDSTREAM URINE was collected in sterile containers and processed immediately.

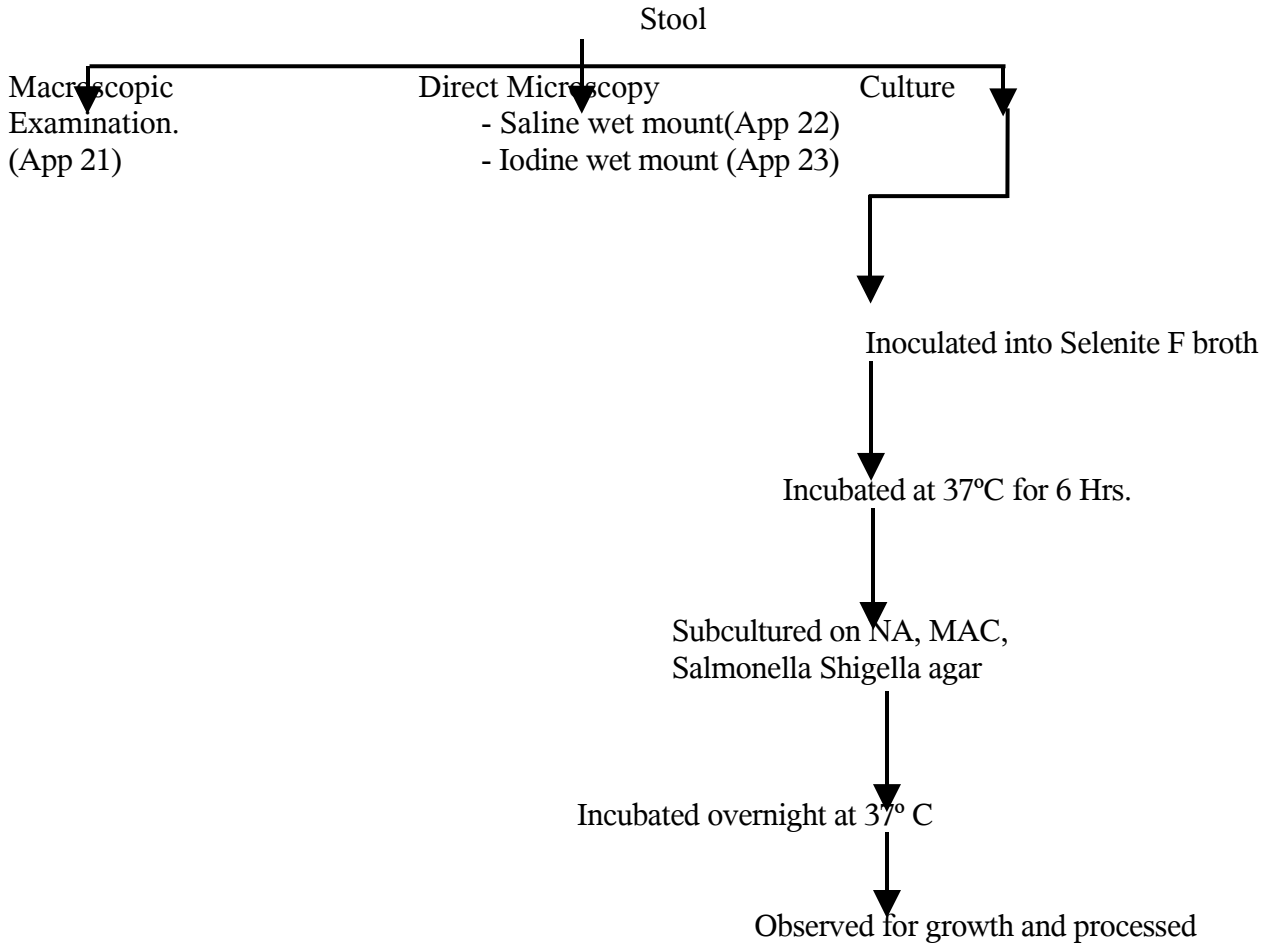


Interpretation of Culture Result

- More than 100,000 (10^5) viable bacteria per ml — significant bacteriruria
- Between 10,000 to 100,000 — doubtful significance — repeat culture — bacteria per ml
- Less than 10,000 bacteria per ml — contaminants, No significant growth

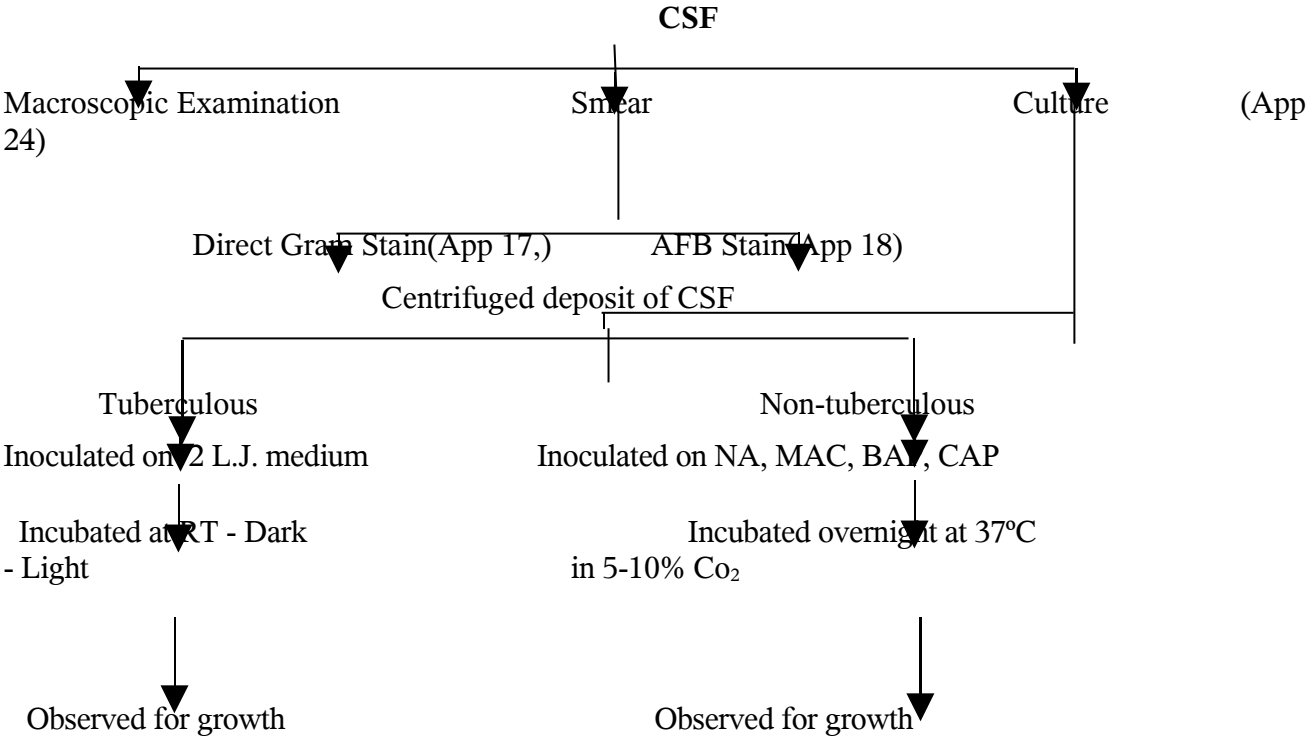
DIARRHOEA (STOOL)

Stool samples were collected in a sterile container and processed as follows.



MENINGITIS (CSF)

CSF was collected with sterile precautions in a sterile container and processed immediately (without refrigeration) as follows.



TB - CULTURE - L.J. MEDIUM

RESULTS

Total number of FUO cases taken up for study - 124

TABLE – 1

Aetiological causes of fever

Infections	*Number of positive*	*Percentage*
Leptospirosis	28	22.6%
Malaria	18	14.5%
Enteric fever	8	6.5%
UTI	7	5.6%
Pneumonia	5	4.0%
Bacteremia	3	2.4%
Brucella	3	2.4%
Tuberculosis	2	1.6%
Mixed infection	2	1.6%
TOTAL	**76**	**61.3%**

Other causes - 48 Percentage : 38.7%

From the above table it is evident that among infectious diseases the commonest cause of fever was found to be leptospirosis followed by malaria. The other significant causes were enteric fever, urinary tract infection and pneumonia.

TABLE - 2

Categorisation of the duration of fever

Disease	***7 days - 2 weeks***	***2 weeks - 1 month***	***> 1 month***
Leptospirosis	14 (50%)	10 (35.7%)	4 (14.3%)
Malaria	14 (77.8%)	3 (16.7%)	1 (5.5%)
Enteric fever	1 (12.5%)	5 (62.5%)	2 (25%)
UTI	5 (71.4%)	2 (28.6%)	0
Penumonia	1 (20.0%)	3 (60%)	1 (20%)
Bacteremia	0	2 (66.7%)	1 (33.3%)
Brucellosis	0	3 (100%)	0
Tuberculosis	0	1(50%)	1(50%)
Mixed infection	2 (100%)	0	0
TOTAL	**37**	**29**	**10**

In majority of cases taken in the study, fever had lasted for 1-2 weeks. Very few cases had fever lasting for more than a month.

TABLE - 3

Sex distribution of fever cases (76)

Disease	No.of Males affected	No. of females affected
Leptospirosis	17 (60.7%)	11 (39.3%)
Malaria	14 (77.8%)	4 (22.2%)
Enteric fever	5 (62.5%)	3 (37.5%)
UTI	2 (28.6%)	5 (71.4%)
Pneumonia	3 (60.0%)	2 (40.0%)
Bacteremia	2 (66.7%)	1 (33.3%)
Brucellosis	3 (100%)	0
Tuberculosis	0	2 (100%)
Mixed infection	0	2 (100%)
TOTAL	**46**	**30**

More men than women reported with fever (except tuberculosis & UTI).

TABLE - 4
Age-wise distribution of fever cases (76)

Disease	***< 20 years***	***21 - 40 years***	***41 - 60 years***	***> 61 years***
Leptospirosis	6 (21.4%)	12 (42.9%)	8 (28.6%)	2 (7.1%)
Malaria	3 (16.7%)	5(27.8%)	8 (44.4%)	2 (11.1%)
Enteric fever	2 (25.0%)	3 (37.5%)	2 (25%)	1 (12.5%)
UTI	1 (14.3%)	4 (57.1%)	2 (28.6%)	0
Penumonia	1 (20.0%)	2 (40.0%)	1 (20.0%)	1 (20.0%)
Bacteremia	1 (33.3%)	2 (66.7%)	0	0
Brucellosis	1 (33.3%)	1 (33.3%)	1 (33.3%)	0
Tuberculosis	1 (50.0%)	1 (50,9%)	0	0
Mixed infection	0	0	2 (100%)	0
TOTAL	**16**	**30**	**24**	**6**

In the present study, most of the cases with fever were between the age group 21 and 60 years.

LEPTOPIROSIS

TABLE - 5

Total number of positive cases - 28, (22.6 %)

Clinical features

Symptoms	*Number*	*Percentage*
Fever	28	100%
Myalgia	21	75%
Conjunctival suffusion	17	60%
Jaundice	13	46%
CNS dysfunction	4	14%
Renal failure	3	11%

Fever, myalgia and conjunctival suffusion were the common clinical features seen in leptospirosis cases.

Jaundice was present in 46% of cases.

TABLE - 6

Investigations and number of positives

Antigen					*Antibody*		
DFM blood	***DFM urine***	***Fontanas staining***	***Culture***	***PCR***	***MSAT***	***MAT***	***Both***
4	Nil	4	2	4	28	26	26

Antigen was detected only in 4 cases, antibody was detected in all the 28 cases.

TABLE - 7
Sero group distribution in MAT positive cases

Sero groups	**No.of positive**	**Percentage (%)**
Icterohaemorrhagiae	14	53.8%

Patoc	4	15.4%
Grippotyphosa	3	11.5%
Hebdomadis	2	7.7%
Louisiana	2	7.7%
Pomona	1	3.8%

Predominant sero group was *Icterohaemorrhagiae* 14 (53.8%)

PARASITIC INFECTIONS

TABLE – 8
Total number of cases screened – 124

Organisms	***Peripheral Blood Smear***	
	Positive	***Negative***
Plasmodium Sp	**18**	-
Filariaworms	-	-
Babesia microti	-	-
Leishmania Sp	-	-
Toxoplasma gondii	-	-

In the peripheral blood smear 18 cases were positive for malaria and other parasites were found to be negative.

MALARIA

TABLE - 9

Total number of positive cases - 18, (14.5%)

Clinical features

Symptoms/signs	***Number***	***Percentage***
Fever with chills	18	100%
Myalgia	13	72.2%
Splenomegaly	13	72.2%
Anaemia	9	50%

CNS dysfunction	3	16.6%
Jaundice	3	16.6%
Renal dysfunction	1	5.6%

All the malaria cases presented with fever and chills. Splenomegaly was present in 72.2% cases.

TABLE - 10

Investigations

Species	***Positive by peripheral smear***	***Positive by PLDH***
Plasmodium vivax	14	14
Plasmodium falciparum	4	4

Malaria was diagnosed by peripheral smear method and by rapid method - PLDH. Both tests were equally effective in the diagnosis of Malaria.

TABLE - 11

Response to Antimalarials

Species	**Response to chloroquine**	**Response to quinine**
Plasmodium vivax	12	2
Plasmodium falciparum	-	4

12 out of 14 cases responded to chloroquine. 6 cases needed quinine therapy. Four cases of falciparum malaria recovered without sequelae.

ENTERIC FEVER

TABLE - 12

Total number of positive cases - 8, (6.5%)

Clinical features

Symptoms	*Number*	*Percentage*
Fever	8	100%
Myalgia	6	75%
Diarrhoea	4	50%
Hepato splenomegaly	3	37.5%
Coated tongue	2	25%

Myalgia was the most common symptom seen next to fever followed by diarrhoea, hepatosplenomegaly and coated tongue.

TABLE - 13

Investigations

Antigen	*Antibody*		*Urine culture*	*Stool culture*
Bl. Culture	**Rapid method**	**Widal**		
1	8	8	Nil	Nil

In this study one case of *S. paratyphi A* was grown in culture. Eight cases of enteric fever were detected by serological method. The rapid method was equally effective as conventional Widal test. Urine and stool cultures were negative for *Salmonella sp*. All the 8 cases positive for antibodies to enteric fever showed rise in titre when repeated after 1 week, seven for *S. typhi* and one for *S. para typhi A*

URINARY TRACT INFECTION

TABLE - 14
Total number of positive cases - 7, (5.6%)

Symptoms

Symptoms	*Number*	*Percentage*

Fever	7	100%
Dysuria	5	71.4%
Vomiting	3	43%
Myalgia	2	28.6%

Among 7 cases of UTI, 5 patients complained of dysuria. Vomiting and myalgia were relatively less common and non-specific. The nature of the fever was commonly high grade and intermittent.

TABLE - 15

Urinary isolates

Organism	***No.of patients***	***Percentage***
E. coli	4	57.1%
Proteus vulgaris	2	28.6%
Staphylococcus aureus	1	14.3%

E. coli was the commonest organism causing UTI, followed by *Proteus vulgaris* and *Staphylococcus aureus*.

PNEUMONIA

TABLE - 16
Total number of positive cases - 5, (4%)

Isolates

Organisms	***No. of cases***	***Percentage***
Klebsiella pneumoniae	3	60%
Staphylococcus aureus	2	40%

Sputum culture identified *Klebsiella pneumoniae* and *Staphylococcus aureus* as causes for pneumonia.

NON-ENTERIC BACTEREMIA

TABLE - 17

Total number of positive cases - 3, Percentage 2.4

Isolates

Isolates	*No.of positive*	*Percentage*
E. coli	1	33.3%
Klebsiella pneumoniae	1	33.3%
Staph. aureus	1	33.3%

E.coli, Kleb. pneumoniae and Staph. aureus were found to cause non-enteric bacteremia.

TABLE - 18
Antibiogram for isolates from UTI, Pneumonia and Bacteremia

Sensitivity Pattern

Organism	*Infection*	*Total No. isolates*	*Cipro floxacin*	*Cepho-taxime*	*Amika-cin*	*Ofloxa-cin*	*Gara mycin*	*Ampi-cillin*	*Norflox acin*
E. coli	UTI	4	3	1	2	0	2	0	1
	Bacteremia	1	1	0	1	1	0	0	-
Klebsiella	Pneumonia	3	2	2	2	3	0	0	-
	Bacteremia	1	0	0	1	1	0	0	-
S. aureus	Pneumonia	2	1	2	1	1	0	1	-
	Bacteremia	1	1	1	1	1	0	0	-
	UTI	1	1	0	1	0	1	0	1
Proteus vulgaris	UTI	2	1	0	1	0	1	1	0
Salmonella paratyphi A	Bacteremia	1	1	1	0	1	1	1	0

Majority of isolates were sensitive to Ciprofloxacin and Amikacin.

BRUCELLOSIS

Total number of positive cases - 3, (2.4%)

TABLE - 19

Antigen	*Case 1*	*Case 2*	*Case 3*
Br. melitensis	1:160	1:80	1:320
Br. abortus	Negative	Negative	Negative

Antibodies to *Br. melitensis* was detected in 3 cases. All the 3 cases positive for antibodies to Brucella showed rise in titre when repeated after 1 week.

TUBERCULOSIS

Total number of positive cases - 2, (1.6%)

TABLE - 20

Symptoms

Symptoms	*Number*	*Percentage*
Fever	2	100%
Cough with expectoration	1	50%
Loss of appetite and weight	1	50%
Evening rise of temperature	1	50%
Altered sensorium	1	50%
Chest pain	-	-
Haemoptysis	-	-

Only one case presented with cough and expectoration.

TABLE - 21
Site and specimen of the lesion

Site	*Specimen*	*Number*
Pulmonary	Sputum	1 (50%)
Extra pulmonary - TB meningitis	CSF	1 (50%)

In both cases, Mycobacterium tuberculosis was demonstrated by Ziehl-Neelsen staining. Both were

culture positive for Mycobacterium tuberculosis.

MIXED INFECTIONS

Total number of positive cases - 2, (1.6%)

TABLE - 22

No.of cases	*Infections*
1	Malaria + Typhoid
1	Malaria + Leptospirosis

One case presented with malaria and typhoid and another case presented with malaria and leptospirosis.

RICKETTSIAL INFECTIONS

TABLE - 23
Weil Felix test

No.of cases	*Antigen*		
	OX 2	*OX 19*	*OX K*
124	Negative	Negative	Negative

Weil Felix test was negative in all 124 cases

DISCUSSION

One hundred and twenty four cases of fever of more than one week duration were taken up for this study. It included 86 males and 38 females. Patients from 12-80 years age group were included in this study. Immuno- deficient patients and patients who were treated already with antibiotics were excluded from this study.

Among the etiologies, infectious disease formed the largest group (61.3%) (Table 1). This was high when compared to other Indian studies by Sharma and Kumari (1992)[99] and Kejariwal D. (2001)[51] where infectious disease formed 50% and 53% respectively. In other studies it was 30% (Larson & Featherstone, 1982)[58], 36% (Petersdorf & Beeson 1961)[87] 40% (Jacoby & Schwartz 1973)[41], 37% (Howard et al. 1977) [38] and 22.5% (Knockaert, 1992) [54]. In an Indian study of FUO by Kucukardaly Y. [56] in 2002 infectious disease formed 59%. This correlates with the present study. The high incidence of infectious etiology in this study was probably due to the fact that this hospital is a tertiary care centre.

Among infectious disease leptospirosis leads the list forming 22.6%. The other causes of infections in this study were malaria 14.5%, enteric fever 6.5%, urinary tract infection 5.6%, followed by pneumonia, bacteremia, brucellosis and tuberculosis (Table 1), two cases were having mixed infections (Table 22).

The fever patterns were namely, continuous, remittent, intermittent and recurrent. It was associated with chills in a few cases. A study in Wardha, India by Jung A[48] in 1999, showed intermittent type of fever more commonly recorded in infectious disease. Duration lasted from 7 days to more than a month. Most of the cases had fever lasting between 7-14 days. Neither the pattern nor the duration was useful in pointing to the diagnosis of FUO (Table 2). General systemic complaints like anorexia and fatigue occurred in over two-thirds of patients and one quarter experienced chills and rigors. But these symptoms were not of any diagnostic significance.

Infectious etiology was detected in 46 males and 30 females in this study. The number of males was slightly higher than the number of females but both TB positive patients were females (Table 3). In Kejariwal D. (2001) [51] study there were 59 males and 41 females out of 100 cases.

In the present study most of the patients fall between the age group 21-40 years (Table 4). An Indian study by Kucukardaly[56] 2002 showed the mean age of the patients to be 39 years which correlates with the present study.

LEPTOSPIROSIS

Leptospirosis topped the list of infections in the present study. It is the most common and important cause of FUO in Chennai (22.6%) (Table 1). An Indian study by Ratnam et al. [93] at Pondicherry in 1983 showed 24% of leptospirosis among FUO cases. This correlates with the present study. The study done at Nagpur, India by R. Angnani[3] 2003 showed 32.73% among FUO patient, which was slightly higher than present study.

The age and sex distribution of patients in this study indicates that leptospirosis is the disease of

occupationally active age group like young to middle age adults with male preponderance. There were 17 males and 11 females among the 28 patients. About 12 (42.9%) belonged to 21-40 years, whereas 6 (21.4%) of patients were less than 20 years and 8 (28.6%) belonged to 41-60 years. 2 (7.1%) paitents were above 60 years (Table 3,4). Marrotto et al[64]., 1997 found that commonest age affected was between 4-14 years.

Myalgia was present in 75% of cases in the present study. Sumathi G [108] (1995) at Madras showed myalgia as a clinical symptoms in 56% cases. Conjunctival suffusion was present in 60% of cases which was higher as compared to 33%, noted by Sumathi G, (1995) in Madras. The study conducted by Sumathi, 1995 showed 55% of cases with jaundice,[108] where as the present gave only 46% positive for jaundice. CNS dysfunction were present in 14% of cases which was lower as compared to 28% in Muthusethupathy[77] study (1995). Fifteen patients (54%) had anicteric leptospirosis. Among the 13 icteric patients, 3 patients had renal failure, 4 patients had CNS dysfunction (Table 5). This study has highlighted the importance played by leptospirosis as a cause of FUO.

Incidence of leptospirosis in FUO during 2003-2004 was 22.6% (Table 1). The DFM positive (4 cases) were cultured, of which leptospires were isolated in culture from 2 cases. They were identified as serogroup *L. icterohaemorrhagiae*. Four cases (14.2%) were positive by DFM and Fontana's staining. All 4 DFM positive cases were PCR positive (Table 6). The percentage of cases detected by DFM was 37.5% in a study conducted by Ramdass et al. [90] (1997). An Indian study at Port Blair in 2001 had a 40.2% sensitivity for DFM.

In the present study 2 cases (7.1%) were positive by culture. The low rate of isolation of leptospires by culture has been documented in legendary studies[27]. Palmer et al[83] (1984) have demonstrated a successful isolation rate of 12% from serologically positive patients. In a study in Erode by Nataraja Seenivasan et al. [79] in 2004, culture was positive in 7 out of 29 patients (24.1%).

Serological test MSAT (a widely accepted screening test for leptospirosis) was positive in 28 cases (100%) (Table 6). Galton et al. [29] (1958) found that *Patoc* strain (killed antigen) used for MSAT was more sensitive, Sumathi et al. [109] (1997) found that MSAT was positive in 39.8% in the year 1996 when they investigated 1461 samples.

The Gold standard serological test for the diagnosis of leptospirosis is taken as MAT. The present study showed MAT to be positive in 26 (92.8%) out of 28 MSAT Positive cases. The other 2 cases diagnosed by MSAT and not by MAT, might have been infected with other serogroups not used in the present study panel of antigens.

The predominant serogroup was found to be *L. icterohaemorrhagiae* (53.8%) followed by *L. patoc* (15.4%), *L. grippotyphosa* (11.5%), *L. hebdomadis* (7.6%), *L. louisiana* (7.6%) and *L. pomona* (3.8%) (Table 7) A study by Ramakrishnan et al. [91] (2003), showed *L. icterohaemorrhagiae* to be the predominant sero group. (20 out of 69 cases - 29%).

MALARIA

Malaria was found to be second most common cause of FUO in this study (14.5%) (Table 1). Majority of them reported with prolonged fever with chills ranging from 7-14 days (Table 2). An Indian study at Calcutta 2001 by Kejariwal[51] had 3 cases of malaria out of 100 cases and Jung A.[48] (1999) at Wardha showed 9% of malaria causing FUO. More malarial infection was seen in males than females (Table 3). Splenomegaly and myalgia was seen in 72%. Anaemia was seen in 9 cases of malaria (50%), 3 patients had CNS dysfunction and jaundice. One case presented with renal dysfunction and jaundice (Table 9).

All the patients were blood smear positive for malarial parasites and also positive for PLDH (Plasmodium Lactate DeHydrogenase enzyme) by rapid method detection. There were 14 cases of *P. vivax* and 4 cases of *P. falciparum* (Table 10). N. Chayani[12] (Orissa 2002) compared the conventional blood smear study with PLDH and their results showed 52.5% and 50.8% respectively. Present study shows conventional blood smear study and rapid malaria test (PLDH) to be equally good. Twelve cases of *P. vivax* responded to Chloroquine while 2 case of *P. vivax* and 4 cases of *P. falciparum* responded to quinine (Table 11). There were 4 cases of cerebral malaria and all of them improved well without sequelae. This study has highlighted the fact that malaria should be kept in mind in patients with prolonged fever.

LEISHMANIASIS

Kala-azar was diagnosed by Kejariwal et al.[51] (2001) in 5 patients (5%) by detecting the presence of L.D. bodies in Bone marrow/splenic aspirate. In the present study, no cases of Kala-azar was detected in 124 FUO cases (No L.D. bodies were seen in the peripheral smear) (Table 8).

BABESIOSIS

Blood Smear was negative for babesia in all cases in the present study (Table 8). So far 100 cases of human infections have been reported world wide. Human infection caused by *Babesia microti* have been reported from North America. So far there has been no report of human babesiosis in India[30].

TOXOPLASMOSIS & FILARIASIS

Toxoplasma gondii and microfilaria were not found in peripheral smear in all 124 cases (Table 8).

ENTERIC FEVER

Enteric fever was the next common cause of prolonged fever and was seen in 8 cases (6.5%) (Table 1). Out of 8 cases 1 had fever of 2 weeks duration, 5 had fever of 2 weeks to 1 month duration and 2 had fever more than 1 month duration (Table 2).

There were 5 males and 3 females in this study (Table 3). Among the age distribution 25% belonged to the age group less than 20 years while 37.5% belonged to age group 21-40 years while 25% belonged to age group 41 to 60 years and only one was above 60 years (Table 4).

Fever in typhoid was the commonest symptom followed by mayalgia 75%, GI disturbances 50%, organomegaly 37.5% and coated tongue 25% (Table 12). A study showed 60% patients had continuous fever and rest had intermittent, remittent or irregular fever with chills and riger. [47] Fever with neuropsychiatric symptoms dominate the picture in India [52]. Where as these symptom was not seen in present study.

The majority of cases responded to Ciprofloxacin(60%) while 40% of cases responded to Ampicillin and Cefotaxime combination. *S. paratyphi A* was isolated in 1 case (12.5%) (Table 13). This correlates with the Indian study of Saxena, S.N. (1966) [98] which showed 3-17% isolation. In India there has been an unusual high rate of isolation of *S. paratyphi A* (46.15%) reported. [113] *S. paratyphi A* isolated in 2002 was 45.45% and in 2003 was 53.33% [68]. A study by Broide E. at Israel (2005)[8] had 9.2% positive by blood culture. All cases improved without sequelae. *S. paratyphi A* was sensitive to Ciprofloxacin, Cephataxime, Ampicillin and Ofloxacin (Table 18). This correlates with an Indian study by S.S. Tankhiwale (2003)[112] which showed 100% sensitive to Ciproflaxacin, Cephotaxime and Ampicillin 66%, Chloramphenicol and Co-trimoxazole showed 72%.

Diagnostic facilities for typhoid fever are frequently not available in rural areas of developing countries, where it is a major problem. The most widely used immunological assay is the Widal test for serum antibodies to O & H antigens. Again this facility may not be available in rural areas. To overcome such problems, the rapid diagnostic methods can be used. The advantage of the rapid test is that the result can be obtained within 15 minutes for a prompt treatment. This study showed that the rapid method for typhoid was found to be equally good as the Widal Test (Table 13).

URINARY TRACT INFECTIONS

Urinary tract infections was the next common cause of prolonged fever and was seen in 7 cases (5.6%) (Table 1). An Indian study by Kajariwal (2001) [51] at Calcutta showed 4% of UTI, which correlates with the present study. Of the 7 patients 5 had fever of 8 days to 2 weeks and 2 had fever of 2 weeks to 1 month duration (Table 2). There were 2 males and 5 females (Table 3). The clinical symptoms seen in these cases were dysuria 71.4%, vomiting 43% and myalgia 28.6% along with fever (Table 14).

E. coli was the commonest organism isolated (57.1%) followed by *Proteus vulgaris* (28.6%) and *Staphylococcus aureus* (14.3%) (Table 15). A study by Kejariwal D. (2001) [51] at Calcutta showed *Klebsiella pneumoniae* 2 (50%), *Proteus sp.* 1 (25%) and *E. coli* 1 (25%) were the causative agents of UTI in FUO cases.

Most of the organisms were sensitive to Ciprofloxacin, Amikacin and Norfloxacin (Table 18). A study by Edward S. Wong (1999) [22] at Virginia showed most of the organism causing UTI was sensitive to Ciprofloxacin, Norfloxacin, Ofloxacin and Sparfloxacin. Ultrasonogram (KUB) was done in all cases but did not reveal any structural anomalies.

PNEUMONIA

Pneumonia was identified in 4% of cases suffering from FUO in this present study (Table 1). One patient had fever of 8 days to 2 weeks, 3 patients had fever of 2 weeks to 1 month and one patient had fever of more than 1 month duration (Table 2). There were 5 cases of pneumonia comprising 3 males and 2 females (Table 3). One patient was in the age group < 20 years, 2 patients were in 21-40 years, one patient was in the age group 41-60 years and one patient was above 61 years (Table 4).

Klebsiella pneumoniae was isolated in 3 patients (60%) and *staphylococcus aureus* in 2 patients (40%) (Table 16). A study by Halm E.A. (2002) [35] had *S. aureus* 64%, *P. aeruginosa* (21%), *Acinetobactor sp.* (6%), *Enterobacter sp.* (9%), *K. pneumoniae* (8%) causing hospital acquired pneumonia. Most of the organism were found to be sensitive to Ciprofloxacin, Cephotaxine and Ofloxacin (Table 18).

NON-ENTERIC BACTEREMIA

Non-enteric bacteremia accounted for 2.4% in this present study (Table 1). A study by Amorn Leelarasamee (2004)[2] at Thailand showed 3.2%. This was correlating with the present study. R.S. Vasan (1998) [120] stated that 5% of bacteremia accounted for FUO. The causative organism were *staph. aureus*, *staph. epidermidis,* Gram negative bacilli causing UTI, pneumonia. Two cases had fever between 2 weeks to one month and one case was more than 1 month (Table 2). They were 2 males and 1 female in this study (Table 3). One isolate of *E. coli* 33.3%, *Klebsiella pneumoniae* 33% and *Staph. aureus* 33.3% was found to be the cause of non-enteric bacteremia (Table 17). A study by Amorn Leelarasamee (2004) [2] at Thailand showed *E. coli* 36.1%, *Staph. aureus* 11.1% and *Klebsiella pneumoniae* 2.8% to be the cause of nonenteric bacteremia. Most of the organisms were sensitive to Cephotaxime, Ciprofloxacin, Ofloxacin and Amikacin (Table 18).

BRUCELLOSIS

Brucellosis was the cause of prolonged fever in 3 cases (2.4%) (Table 1) Out of 3 cases 2 patients had animal contact and presented with intermittent fever, chills and arthritis of 1 month duration. Remaining one case did not have any animal contact. A study by Mudaliar S. (2003) [75] reported that 8.5% were animal handlers. All three cases showed antibodies to *Br. melitensis* (Table 19). CMC, Vellore study by Grace Koshi[32] showed 20 cases of brucellosis over a period of 10 years in 1967. Another Indian study by Kucukardaly Y. (2002) [56] found 13% of brucellosis were causing FUO.

TUBERCULOSIS

Tuberculosis constituted 1.6% of the cases of prolonged fever (Table 1). An Indian study by Jung (1999) [48] had 5.2% of tuberculosis fever and Kucukardaly Y. [56] (2002) had 12% of tuberculosis in FUO cases. The fever was commonly low grade in nature and lasted for more than a month in one case and 2 weeks duration of fever in TB meningitis case. Cough, anorexia, loss of appetite, weight and lymphnode enlargement was common associated symptoms (Table 20). Among 2 cases, one case was found to be pulmonary tuberculosis and the other was TB meningitis.

AFB smear and culture were positive in both cases (Table 21). A study by N. Hemvani[37] at Indoor (1999) showed 63% of sputum samples were smear positive. In this study, Mantoux test was positive in one case. ESR, Chest X-ray aided in the diagnosis. CT scan, MRI helped in diagnosis of TB meningitis case. TB meningitis responded dramatically to anti-tuberculous treatment without sequelae. Since immunosuppressed patients were excluded from this study, the incidence of tuberculosis may have been very low.

MIXED INFECTION

Two cases had mixed infection in the present study. One case was malaria with leptospirosis and another case was malaria with typhoid (Table 22). Ramakrishnan et al. (Madras, 1999) [92] had reported 22% incidence of coinfections in leptospirosis. The most common associations were with enteric fever 17% and malaria 5% cases.

RICKETTSIAL INFECTION

Weil-Felix test was found to be negative in all cases in the present study (Table 23). A study by Amorn Leelarasmee (2004)[2] at Thailand showed 7.5% of Scrub typhus.

AMOEBIASIS

Present study showed no trophozoites or cyst in saline wet mount or iodine wet mount. Thirty percent of amoebiasis has been reported in Maharashtra, Tamilnadu and Chandigarh. Twenty percent of cases reported from Andhra Pradesh, Kerala, Punjab and West Bengal[61].

OTHER CAUSES

In this study, 48 cases were negative for all the above tests. Out of 48 cases, 3 cases were diagnosed to have clinical malaria and responded to antimalarial treatment, 4 cases were clinically diagnosed as pulmonary tuberculosis and responded to anti-tuberculosis therapy. The remaining undiagnosed 41 (33%) cases may come under viral / fungal infections or may be due to other causes like neoplasm, collagen diseases, miscellaneous and undiagnosed. This is relatively less in contrast to the study by deKleijn E.M. (1987)[19], Kejariwal 1998 – 2000[51] and Kucu Kardaly 2002[56] where the percentage of undiagnosed cases alone (other than infections, neoplasm, collagen disease and miscellaneous) were about 29.9%, 14% and 21% respectively.

SUMMARY

- A total of 124 FUO cases were taken up for the study.
- Microbial infections (Bacterial and Parasitical) were seen in 61.3% of FUO cases.
- Leptospirosis (22.6%) was the most common infection followed by malaria (14.5%), enteric fever (6.5%), UTI (5.6%), pneumonia (4%), Brucellosis and Bacteremia (2.4%) and tuberculosis (1.6%)
- Mixed infections were seen in 2 cases, which comprised of malaria with leptospirosis and typhoid.
- Most of the cases had fever lasting for 1-2 weeks (37 cases) 50%
- Males were infected more than the female population (46:30)
- Majority of the cases fell into the age group 21 to 60 years.
- Myalgia and conjuctival suffusion were the common clinical features seen in the leptospirosis cases. From blood in 2 cases of leptospirosis, *L.icterohaemorrhagiae* was grown. In 4 cases leptospirosis were seen by Fontana's staining and DFM. In the same cases leptospiral antigen was identified using PCR. Antibodies were detected by MSAT in 28 cases and by MAT in 26 cases. *Icterohaemorrhagiae* (53.8%) was the predominant serogroup identified by MAT followed by *Patoc* 15.4%, *Grippotyphosa* 11.5%, *Hebdomadis* and *Louisiana* 7.7 % and *Pomona* 3.8%.
- All malarial cases presented with fever and chills followed by myalgia and splenomegaly. Both the conventional peripheral smear method and PLDH (rapid method) were equally effective. All 18 cases were positive by both methods. Twelve cases infected by *P.vivax* responded to **C**hloroquine and two to Quinine. Four cases of *P.falciparum* responded to quinine.
- Myalgia was the most common symptom seen in cases with typhoid followed by diarrhoea, hepatosplenomegaly and coated tongue. *S.paratyphyi A* was grown in culture in one case. Eight cases were picked up by the conventional Widal test and the rapid salmonella antibody detection test for the presence of antibodies against *S.typhi* and *S.paratyphi* A showing both tests to be equally good.
- Dysuria (71.4%), vomiting (43%) were the clinical symptoms seen in these patients. *E. coli* 4 (57.1%), *Proteus vulgaris* 2 (28.6%) and *S. aureus* 1 (14.3%) were the etiological agents causing UTI.
- Only 5 cases had Pneumonia where *K. pneumoniae* 3 (60%) and *S. aureus* 2 (40%) were the causative agents.
- The etiological agent isolated from 3 cases of bateremia were *E. coli, K. pneumoniae* and *S. aureus* one each.
- Ciprofloxacin was the drug of choice followed by Amikacin and Gentamycin for the bacterial pathogens isolated.
- Brucellosis was identified in 3 patients, all of them had antibodies to *Br. melitensis* and two of them were animal handlers.
- Mycobacterium tuberculosis was demonstrated in smear study (Sputum 1 and CSF 1) from 2 patients.

Typhus fever, Babesiosis, Filariasis, Amoebiasis, Kala-azar and Toxoplasmosis were not seen in the present study.

CONCLUSION

- Infections (61.3%) remain the most important cause of FUO in India, confirming the trends found earlier in other studies.
- Leptospirosis (22.6%) followed by malaria (14.5%) and enteric fever (6.5%) were the common infections diagnosed.
- *L.icterohaemorrhagiae* was the predominant sero group.
- The rapid diagnostic tests used in comparison with the conventional methods in the diagnosis of malaria and enteric fever were equally good.
- The rapid tests were simple, sensitive and easy to perform and could be recommended for early diagnosis in both urban and field studies.
- *E. coli, Klebsiella pneumoniae, Staph. aureus* and *Proteus vulgaris* were the etiological agents of UTI, pneumonia and bacteremia.
- In majority of isolates the drug of choice were Ciprofloxacin followed by Amikacin.
- Babesiosis, Leishmaniasis, Typhus fever, Toxoplasmosis, Filariasis and Amebiasis were not detected in the present study.
- This study indicates that with the appropriate use of new sero diagnositic and newer microbilogical methods will result in increased number of cases being diagnosed early and appropriate treatment can be given.
- The role of viruses and fungi have not been touched upon in this study. Further studies of other etiological agents will help in diagnosing the other causes of infectious fever.

PROFORMA

Name Age/Sex IP No. DOA Occupation

Symptomatology
Fever
a) Duration

b) Chills/Rigor
c) Continuous / intermittent
Haemotypsis
d) Sweats

Gastro Intestinal Tract (GIT)

a) Abdominal pain
b) Vomiting
c) Constipation
d) Loose stools
e) Burning micturition
f) Haematuria
g) Jaundice

Cardio Vascular System (CVS)

a) Chest pain
b) Dyspnoea
c) Palpitations
d) Giddiness
e) Edema feet

Respiratory System (RS)

a) Cough with expectoration
b) Wheeze
c) Hemoptysis

Central Nervous System (CNS)

a) Head ache
b) Seizures
c) Altered sensorium
d) Loss of consciousness

Others

a) Body ache
b) Joint pain
c) Rashes
d) Epistaxis
e) Facial pain

GENERAL

a) Loss of appetite
b) Loss of weight
c) Drug intake
d) Tick/Animal bites
e) History of Tuberculosis, Diabetes mellitus, Hyper tension
f) Smoking/Alcohol
g) History of contact with animal

EXAMINATION

a) Temperature chart
b) Anaemia
c) Conj. sufficion
d) Pallor
e) Cyanosis
f) Icterus
g) Clubbing
h) Nodopathy
i) Edema feet
j) Rashes
k) Joint swelling
l) Purpuric spots
m) Oral cavity
n) Nose
o) Ear
p) Sinus tenderness
q) Bony tenderness
r) Pulse rate
s) Blood pressure
t) Respiratory rate
u) Cardio vascular system
v) Respiratory system
w) Central nervous system
x) Per abdomen

Investigations

- Microbiological
- Clinico-pathological
- Biochemical
- Radiological

Samples screened	**Tests done**
Blood/Serum/Plasma	- Blood culture - Cultivation of Leptospira - DFM - for leptospira - Fontanas Staining - PCR - for leptospira - Peripheral blood smear for malarial parasite filarial worms

	babesia leishmania toxoplasma gondii - Rapid test for plasmodium - PLDH - Serological tests MSAT/MAT Widal agglutination test Rapid slide agglutination test for typhoid Brucella agglutination test Weil-felix test
Sputum	Macroscopic Examination Smear Gram stain AFB stain Culture Tuberculous Non-tuberculous
Urine	Direct Microscopy Gram stain DFM - for leptospira Culture Bile salts Bill pigments
Stool	Macroscopic Examination Direct Macroscpy Saline Wet mount Iodine Wet mount Wet mount/Iodine mount Culture
CSF	Macroscopic Examination Gram stain AFB stain Culture Tuberculous Non-tuberculous

Other investigations

Clinico pathological	TC, DC, ESR Biopsy Bone marrow study
Biochemical test	LFT, Renal function test
Radiological	X-ray chest, spine, ultra sound, CT Scan, MRI Scan

Skin test

Mantoux test

APPENDIX

1.Standard protocol[24]

GRAM NEGATIVE ORGANISMS

Organism	Motility	Indole	MR	VP	Cit	TSI H_2S	Urease	PPA	
E.coli	+	+	+	-	-	-	-	-	
Klebsiella pneumonia	-	-	-	+	+	-	+	-	
Proteus vulgaris	+	+	+	-	-	+	+	+	
Salmonella paratyphiA	+	-	+	-	-	-	-	-	

GRAM POSITIVE ORGANISMS

Organism	Cata-lase	Coagu-lase	MR	VP	Argi-nine	Orni thine	Ure-ase	Mal-tose	Lac-tose	Suc-rose	Man-nitol	Dman -nose	Xylose	Arabi-nose	Raffi-nose
Staph. aureus	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-

MR – METHYL RED
VP – Voges – Proskauer
Cit – Citrate
TSI – Triple Sugar Iron Agar
PPA – Phenylalanine deaminase

2. Dark Field Microscopy (DFM)[26]

To 5 ml of blood, 0.5 ml of 1% Sodium Oxalate added

↓

Centrifuged at 1000g for 15 mts.

↓

10 µl of plasma placed on slide and coverslip placed over it

↓

Observed for motility of leptospires under DFM

↓

Leptospires seen -report as DFM positive

No Leptospires seen

↓

Plasma centrifuged at 3000-4000g for 20 mts.

↓

Sediment examined for Leptospires

3. Fontan's Staining

Fixative: Acetic acid 1ml, Formalin 2ml, distilled water 100ml.

Mordant: Phenol 1gm, tannic acid 5gm, distilled water 100ml.

Ammonical Silvernitrate: 10% ammonia to 0.5% solution of silver nitrate in distilled water was added until the precipitate formed just dissolves. Now more silver nitrate solution were added drop by drop until the precipitate returns and does not redissolve.

Procedure :

The slide was flooded three times, 30 seconds each time with the fixative.

↓

Fixative was washed with absolute alcohol and allowed to act for 5 min.

↓

Excess alcohol was drained and burned the remainder until the slide was dry.

↓

Mordant were poured and heated till steam rises and allowed to act for 30 seconds.

↓

Washed with distilled water and dried.

↓

Treated with ammonical silver nitrate, heated till steam rises for 30 secs till the film becomes brown in colour.

↓

Washed with distilled water, air dried, viewed under oil immersion

4. LEPTOSPIRA CULTIVATION

EMJH BASE

EMJH Base Ingredients (Difco Lab, U.S.A.)	Gram / litre
Sodium Phosphate diphasic	1 gm
Pottassium Phosphate monobasic	0.3 gm
Sodium Chloride	1 gm
Ammonium Chloride	0.25 gm
Thiamine	0.005 gm
D. Water	1000 ml

EMJH ENRICHMENT

The enrichment Bovine serum albumin (BSA), one gram of BSA per 10ml of EMJH medium at final concentration of 10% was filtered through a membrane (Pore size 0.22µ) and was added as enrichment after cooling.

EMJH LIQUID MEDIUM (BUFFERED SALT BASE + ENRICHMENT)

- 2.3 gm of EMJH base dissolved in 900ml of triple D. Water
- PH adjusted to 7.5
- Autoclaved at 15 lbs for 15 mts.
- 10% (100ml) enrichment was added to 900ml medium aseptically
- Distributed in screw capped tubes.

EMJH SEMISOLID MEDIUM (0.2 % Agar + liquid medium)

- 0.2% agar was added to the EMJH base liquid medium

SELECTIVE AGENT[46]

- 10mg of 5 – flourouracil (5FU) added to 5ml of D. Water
- 0.1 to 0.2ml of 0.1N NaoH was added
- heated gently to dissolve 5 FU completely
- PH adjusted to 7.4 to 7.6
- Volume was made up to 10ml with D. Water
- Sterilized by membrane filter (Pore size 0.45 µ)
- 1 ml was added to 100 ml of EMJH medium to get the final concentration of 100 ugm per ml.

5. PCR – LEPTOSPIRA[69]

- Amplification of DNA was performed in a total volume of 50 µl reaction mixture
- The reaction mixture contains 5 µl of 10x buffer (50 mM Kcl, 20 mM $mgcl_2$, 10mM TrisHcl, PH 8.3)
- The freeze – dried primers G1 & G2 were mixed with nuclease free water to prepare stock solutions. From this primer stock solution 100 mM each were added to the reaction mixture.
- The four deoxy nucleotide Triphosphate 100 mM of dNTP each of ATP, GTP, CTP, TTP (totally 400 mM) was used.
- To this reaction mixture, 3 µl Taq DNA polymerase and 5 µl of sample were added with

required amount of distilled water (31µl) to make up the volume to 50 µl.

- ❖ Amplification was carried out in MJ research. DNA thermal cycler for 34 cycles with intial denaturation of DNA for 6 ½ minutes at 94°C, then subsequently with each cycle consisting of denaturation of DNA for 2 mts at 94°C, annealing of the primer for 1mts at 55°C and elongation of chain for 2 mts at 72°C. After the completion of necessary clycles, a warming up period of 7 mts was given.
- ❖ The PCR products were analysed by gel electrophoreris in 1.5% agarose (sigma) in Tris borate buffer of PH 8.3[119]
- ❖ After the electrophoreris was completed, the gel is viewed in UV- Transillumenator where PCR products and Molecular weight marker are seen as bright fluorescent adducts due to binding adduction of Ethidium bromide to DNA[44]

6. INGREDIENTS OF LEISHMAN STAIN

- 0.15 gm of tablet
- Dissolved in 100 ml of absolute methyl alcohol.

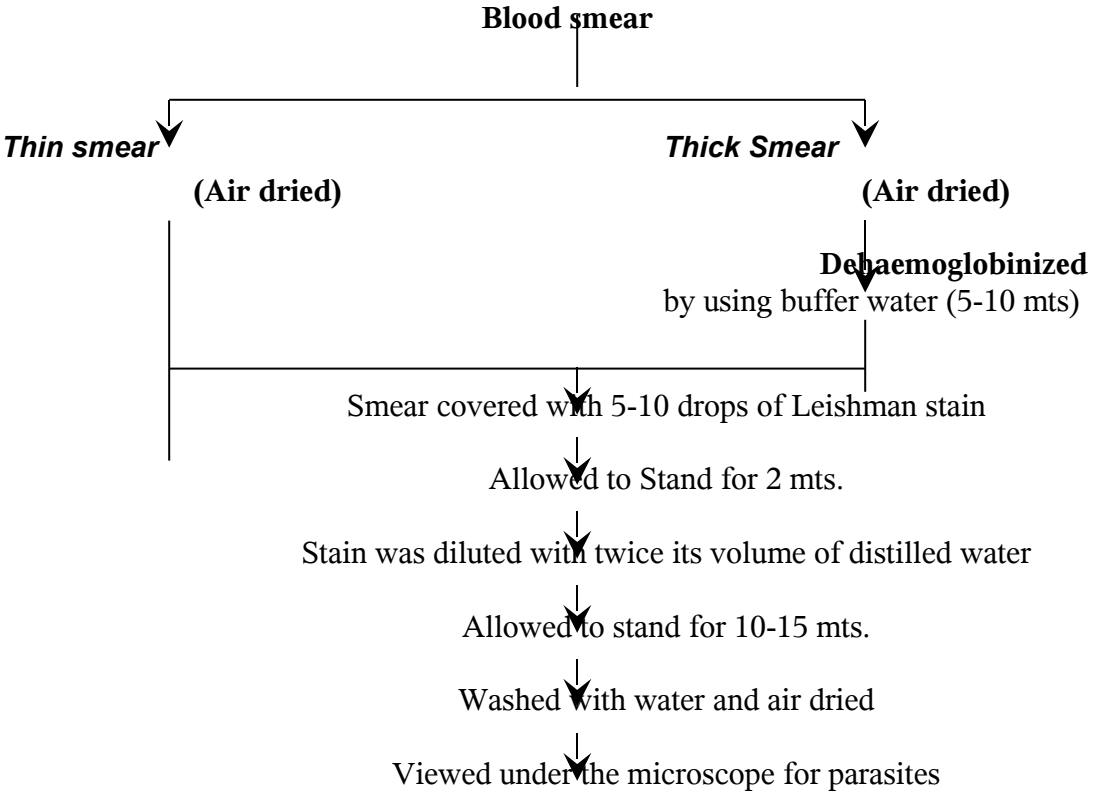
7. BLOOD SMEAR

Thin Film

One drop of blood was placed over glass slide and with the help of spreader at 45°angle, thin smear was made.

Thick Film

One drop of blood was placed on the same slide of the other corner. By using corner of the spreader circular thick smear was made.

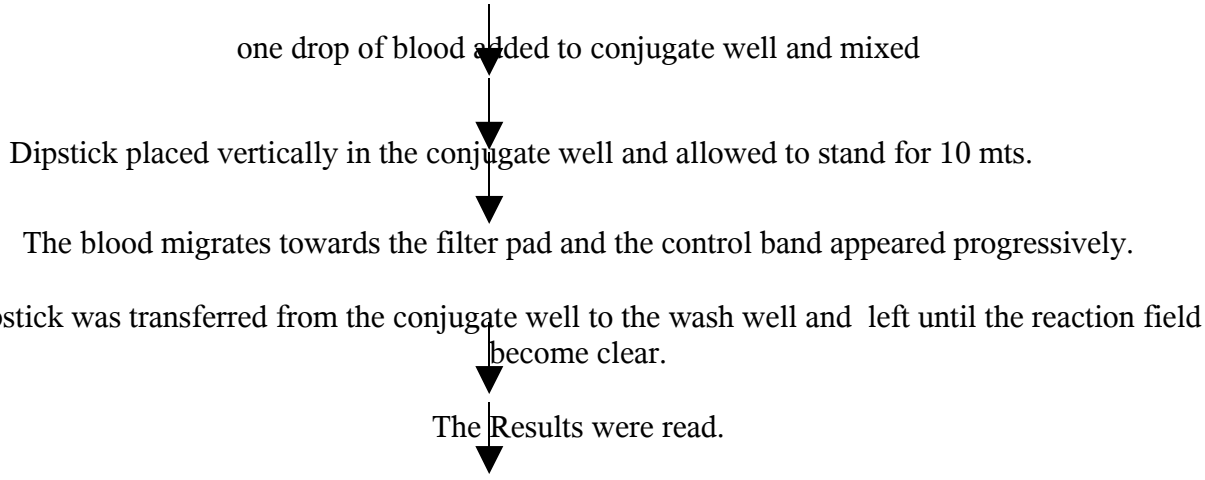


8. DEC – PROVOCATION TEST

Diethyl carbamazine (DEC) provokes the microfilaria to come in the peripheral blood even during the day time. DEC is given in 2mg /kg body weight as a single dose and after about an hour blood sample was collectd for detection of microfilaria.

9. RAPID TEST FOR MALARIA (OptiMAL)

- One drops of buffer was added into the conjugate well
- 4 drops into the wash well



10. MACROSCOPIC SLIDE AGGLUTINATION TEST (MSAT) [67]

a. 8µl of PBS were placed to all the depression of the slide
b. 12µl of heat killed pooled antigen was added
c. 5µl of patients serum was added.

Plate rotated gently for 4 mts. by using a rotator (180 rpm)

↓

Observed for agglutination macroscopically and confirmed by DFM

MICROSCOPIC AGGLUTINATION TEST (MAT)

Sera were diluted 1 in 10 ratio (Tube)

↓

25µl of PBS (PH 7.2) were added in wells

↓

Sera were diluted by doubling dilution

↓

25µl of live antigen were added

↓

Mixed gently and kept at R.T. for 2 hrs.

↓

One drop from each well was examined in

DFM from lowest dilution onwards

↓

Looked for agglutination.

11. WIDAL AGGLUTINATION TEST

Doubling dilution of serum was done
For O, H, AH and BH from 1:25 upto 1:400

↓

Equal Quantity of corresponding antigen were added

↓

Incubated

← 2 to 4 hrs at 37°C
Read after standing on the bench
For half an hour for 'H' agglutinins.

↓

Results were read for 'O' agglutinins

→ 4 to 6 hrs at 37°C
Overnight refrigeration

↓

13. RAPID SLIDE TEST FOR TYPHOID (SPAN DIAGNOSTIC)

- One drop of undiluted serum placed in 1st four circles (1 to 4)
- One drop of positive control serum in last two circles (5,6)
- One drop of antigens O, H, A(H) and B(H) was added in circle 1,2,3 and 4 respectively and 'O' antigen in circle 5 and any one of the 'H' antigen (H, AH, BH) in circle 6.
- Mixed with applicator stick.
- Slide was rocked and observed for agglutinations.

14. BRUCELLA AGGLUTINATIN TEST

Sera was diluted by doubling dilution as 1:20 upto 1:640 in 2 sets
(*Br.melitensis* and *Br.abortus*)
Equal Quantity of corresponding antigens were added
Incubated at 37°C for 48 Hrs.
Results were read.

15. WEIL – FELIX TEST

Doubling dilution of serum was done
For OX2, OX19 and OXK from 1:20 upto 1:1280
Equal quantity of corresponding antigens were added
Incubated at 37°C for 2 Hrs.
Followed by
Overnight incubation at 4°C
Results were read.

16. MACROSCOPIC EXAMIANTION (SPUTUM)

Sputum was examined for colour - Red (blood)
Brown (altered blood)
Yellow green
Whitish or yellow

Consistency - Thick sputum

Thin sputum

17. GRAM STAIN (Common for Blood, Sputum, Urine)

- Smear was covered with methyl violet – 1min
- Washed with water
- Grams Iodine was added – 1min
- Washed with water
- Decolourised with acetone and washed with water
- Dilute Carbol fuchsin was added – 1min.
- Washed with water, air dried and viewed under oil immersion.

17. a Direct microscopy (sputum)

Cells per field	Interpretation

*	10 or more squamous epithelial cells	-	Contaminated with saliva
*	25 or more leucocytes	-	Infection
*	Leucocytes predominantly neutrophils	-	Pyogenic infection
*	Presence of mononuclear leucocytes	-	Mycobacterial infection
*	Bacteria seen	-	To be cultured

18. AFB STAIN

- Smear was covered with strong carbol fuchsin
- Kept for 7 – 10 mts with intermittent heating.
- Washed with water
- 2% gabborts methylene blue was added and allowed to react for 3 mts.
- Washed with water, air dried.
- Viewed under oil immersion.

19. DIRECT MICROSCOPIC EXAMINAITON (URINE)

. Pus cells along with RBC - Haemorrhagic cystitis

. Pyuria without bacteria - Renal tuberculosis

Ciprofloxacin	5 mcg	15	16-20	21
Cephotaxime	30 mcg	14	15-22	23
Amikacin	30 mcg	14	15-16	17
Ofloxacin	5 mcg	12	13-15	16
Garamycin	10 mcg	12	13-14	15
Ampicillin	10 mcg	13	14-16	17
Norfloxacin	10 mcg	12	13-16	17

. Tubular epithelial cells glomerulo nephritis (or)
Urinary cast
tumour

20. DFM - URINE

A drop of urine / centrifuged deposits was placed on the glass slide and coverslip placed over it and viewed under DFM for typical motility of leptospires.

21. MACROSCOPIC EXAMINATION OF STOOL

Macroscopic examination of stool was done. Consistency, colour, odour and presence of blood and mucus was noted.

22. SALINE WET MOUNT

A drop of saline was placed on the slide, a small portion of stool was picked and mixed with saline. The cover slip was placed over it and examined under the Microscope.

23. IODINE WET MOUNT

drop of Iodine was placed on the slide, a small portion of stool was picked and mixed with Iodine. The cover slip was placed over it and examined under the microscope.

24. MACROSCOPIC EXAMINATION (CSF)

* turbid, yellow colour - Indicates presence of xanthochromic, result from previous cerebral haemorrhage

* Blood stained - indicates trauma

25. DRUGS USED FOR ANTIBIOGRAM

Antimicrobial agent	Disc content			
		Resistant mm or less	Intermediate mm	Sensitive mm or more

L.J. MEDIUM

Pottassium dihydrogen phosphate	-	2.4 gm
Magnesium Sulphate	-	0.24 gm
Magnesium Citrate	-	0.60 gm
Asparagine L	-	3.60 gm
Glycerol	-	12 ml
D. Water	-	600 ml
Whole egg	-	1000 ml
Malachite green 2%	-	20 ml

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